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Comparison of Four Methods of Measuring Blood Flow

by

Andrew Weber

**A thesis submitted to the Faculty of Graduate Studies and Research in Partial
Fulfilment for the degree of Master of Science**

**Department of Physics
Carleton University
Ottawa, Ontario
November 1991**

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**The undersigned recommend to the Faculty of Graduate Studies
and Research acceptance of the thesis**

"Comparison of Four Methods of Measuring Blood Flow"

submitted by Andrew Weber, B.Sc.

**in partial fulfilment of the requirements for
the degree of Master of Science**

A handwritten signature in black ink, appearing to read "P. J. W. for L. Gering", is written over a horizontal line.

Thesis Supervisor

A handwritten signature in black ink, appearing to read "P. J. W.", is written over a horizontal line.

Chairman, Department of Physics

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November 1991

Abstract

Four methods of measuring blood flow have been calibrated and compared in vivo and in vitro with respect to their ability to accurately measure perfusate flow through a vessel; transit time ultrasound, square wave electromagnetic, thermal pulse decay and radioactive tracer microspheres. Measurement artifact and accuracy as a function of perfusate temperature, type of perfusate, coupling medium, type of flow (pulsatile, constant), vessel size, vessel material and vessel orientation are considered for both cannulating and perivascular electromagnetic and ultrasonic flow probes.

For the ultrasonic flow probe, the accuracy of flow measurement was found to be independent of most of these parameters however it was found that the flow probe needed to be calibrated for each of the different perfusates, and depended on vessel orientation in the ultrasonic window. The signal strength was found to be dependent upon the type of acoustic couplant and vessel type.

The accuracy of flow measurement by the electromagnetic flow probe was independent of the perfusate temperature and type of flow, but was dependent upon the vessel diameter, orientation of the vessel, and type of perfusate.

The radioactive tracer microsphere technique and thermal pulse decay probes were studied in vivo using an isolated, autoperfused and in situ pig kidneys. The accuracy and performance of these techniques were determined in reference to the ultrasonic and electromagnetic flow probes and are described.

In our in vivo and in vitro studies, the ultrasound flow probe was found to be the most stable and accurate of these devices. Its overall performance and versatility were also superior.

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Nomenclature

B	Magnetic Field Strength
v	Fluid Velocity
E	Electric Field
j_w	Current Density in the Wall
j_m	Current Density in the Medium
σ_w	Electrical Conductivity of the Medium
σ_m	Electrical Conductivity of the Wall
σ_b	Electrical Conductivity of Blood
σ_s	Electrical Conductivity of Saline
σ_{layer}	Electrical Conductivity of the Fluid Boundary Layer
V_w	Potential in the Wall
V_m	Potential in the Medium
r	Radius
a	Inner Wall Thickness
A	Outer Wall Thickness
Q	Volume Flow
T	Transit Time of an Acoustic Pulse
c	Velocity of Ultrasound in the Medium
U	Unamplified Signal Strength
A_{tr}	Transmitter-receiver Area
L	Distance between Transducers
w	Width of Wide Ultrasonic Beam
h	Height of Wide Ultrasonic Beam
ω	Frequency of Ultrasound
α, θ	Angles of Ultrasound Beam and Flow
ρ_t	Density of Tissue
ρ_{bl}	Density of Blood
c_t	Specific Heat Capacity of Tissue
c_{bl}	Specific Heat Capacity of Blood
Θ_t	Thermal Distribution above the Background Temperature
k_t	Thermal Conductivity of Tissue
k_{bl}	Thermal Conductivity of Blood
W	Perfusion in Tissue
P_b	Power Deposited in the Thermal Pulse Decay Probe
τ'	Duration of Heating Pulse
c_a	Arterial Concentration of Microspheres
ε	Trapping Efficiency
A_s	Activity of Sample
A_R	Activity of Reference
p	Pressure
K_B	Bernoulli's Constant

1 Introduction

This thesis has three main objectives. The first is to examine the principle of operation of the four methods used to measure blood flow; the square wave electromagnetic flow probe, the transit time ultrasonic flow probe, a thermal pulse decay probe and the radioactive tracer microsphere technique. Second, this work will examine the measurement artifact associated with each of these under various conditions such as different types of flow, (pulsatile, constant), type of perfusate and perfusate temperature. A knowledge of how a probe or technique responds in vitro with the variation in a particular parameter such as temperature, will provide an understanding of how the device is affected by similar changes in vivo. Third, this work will compare and contrast these methods with respect to their ability to measure flow and their stability, accuracy and overall versatility.

Blood flow (ml/min) is defined as the volume of blood passing through some surface per unit of time. Blood perfusion (ml/min/g) on the other hand, is the total blood flow per unit mass of the tissue. A knowledge of blood flow/perfusion is critical in many areas of medical sciences. Blood flow is a principal mechanism for the removal of heat during local hyperthermia^{1,2,3,4,5,6}. Alterations in local and systemic blood flow during therapeutic heating modifies the temperature distributions achieved in the tissue/tumour environment.

Many tumours have a necrotic centre and are actively growing only on the peripheral layer. Under physiological heat stress the well-vascularized normal tissue can efficiently transport away heat by increasing their flow of blood. Many tumour tissues are incapable of altering their blood flow in order to remove excess heat. Differential blood flow response between normal and neoplastic tissue, results in preferential tumour heating. Since it is difficult to limit the delivery of heat solely to the tumour tissue, it is essential to understand the basal blood flow, as well as the affects of hyperthermia on blood flow⁷.

Blood flow is also responsible for blood gas levels (pO_2 , pCO_2), nutrient and waste transport and can influence tissue pH⁸. These factors can drastically alter tissue (normal and malignant) response to various treatment modalities. It has been shown that tissue pH is a major factor in the efficacy of hyperthermia and cytotoxic agents⁸.

The delivery of chemotherapeutic agents is hindered by the inherently poor flow found inside bulky neoplastic tissues. Increasing the blood flow to the tumour through hyperthermia or with the administration of drugs, will in turn increase the quantity of chemotherapeutic agent delivered to the tumour with a concomitant clinical benefit.

Accurate knowledge of blood flow is an essential part of any theoretical model which might be used for hyperthermia treatment planning or for the determination of the spatial temperature distribution from isolated measured temperatures. One such model is the bioheat transfer equation, described in section 2.3. For a point source of heat embedded in tissue, it can be written as,

$$\rho_t c_t \frac{\partial \Theta(r,t)}{\partial t} = k_t \nabla^2 \Theta(r,t) - W c_b \Theta(r,t) + P_b \delta(t) \delta(t-\tau') \quad [1]$$

where $\Theta(r,t)$ is the thermal distribution above the background temperature, k_t , c_t and ρ_t are the thermal conductivity, heat capacity and density of the tissue respectively. The P_b term is the power input of the thermal point source. The effect of blood flow is contained in the perfusion term, W .

In order to evaluate equation [1], it is critical that blood flow measurements are accurate. For both hyperthermia or chemotherapy treatments, it is necessary to know how blood flow changes and what is the basal or normal response. It is therefore crucial that the limitations and optimal conditions of use are understood for techniques which measure blood flow. When should a type of measurement be used? When should it not? These are the quantities addressed in this work.

In this report, four methods for measuring blood flow are studied. These are: an electromagnetic blood flow probe, a transit time ultrasound probe, a thermal pulse decay probe and the radioactive tracer microsphere technique.

Blood is electrically conductive. If a magnetic field is applied across the vessel, a voltage, perpendicular to the field and proportional to the flow, will be induced. The electromagnetic flow probe provides the magnetic field and using two diametrically opposed sensing electrodes, is capable of measuring the induced potential.

The ultrasonic flow probe is based on the principle that the transit time of an acoustic wave travelling through a flowing medium will be influenced by the flow. The difference between the upstream and downstream transit times can be related to the flow of the medium.

The principle of pulse decay is employed in the thermal probe used in this investigation. If a heating pulse is electrically generated in a small probe volume, then the rate at which the heat decays away from the probe is related to the flow of perfusate around it.

The radioactive tracer microsphere technique utilizes the principle that if an indicator is injected into the blood stream, then the amount of indicator present in a specific organ is related to the flow through the organ. Microspheres are injected and trapped in the organ. By counting the activity (number of microspheres) trapped in the organ, the flow through the organ may be determined.

These four methods differ not only in technique but also in the types of flow that they are able to measure. The ultrasonic and electromagnetic flow probes are capable of measuring only arterial and venous flows. The microsphere trapping and thermal diffusion probe can be used to measure either arterial flow or local tissue blood perfusion.

The microsphere method can only measure the flow at discrete points in time, the time at which the microspheres are injected. The number of flow measurements is limited to the number of available isotopes and resolution of the detector system. The other three techniques, ultrasonic, electromagnetic and thermal, allow one to measure blood flow and changes in blood flow continuously.

In addition to in vitro experiments, it is important to verify performance in vivo. To this end, the swine kidney was chosen as a model for this study. The kidney is an ideal model in that it has a single input and a single output, (neglecting the small contribution by the ureter). This makes it possible to monitor the total flow through the organ by measuring the flow through the renal artery or vein. The electromagnetic or ultrasonic probes may be used here. Microspheres may be injected into the kidney through the normal circulation and the thermal pulse decay probes can be inserted into the tissue of the kidney with relative ease. In this way all of the techniques may be tested and compared against one another in vivo. Surgically, the organ is relatively easy to locate and isolate. In addition, the swine kidney is morphologically similar to the human kidney⁹. Since radioactive tracer microspheres are used, the relatively small size of the organ makes it possible to dissect and subsequently analyze the gamma spectrum emitted by the microspheres trapped in the organ in a reasonable amount of time. The high trapping efficiency of the kidney, discussed in section 2.4, makes it an ideal organ to use in combination with tracer microspheres. Each kidney receives approximately an eighth of the cardiac output and hence the expected flow rates 0 to 300 ml/min or 0 to 3 ml/min/g are within the range of measurement of the chosen techniques.

Since all of the devices studied in this investigation will eventually be used in vivo, the physical properties associated with the animal dictate some of the conditions under which the instruments should be tested. In particular, the core body temperature of the pig is 39.0°C and is not expected to deviate by $\pm 4^\circ\text{C}$ in these experiments. The devices will therefore be calibrated for media temperatures from 35.0 to 45.0°C, adequately covering the anticipated range.

2 Theory of Operation

2.1 Electromagnetic Blood Flow Probes

One commonly used instrument for the measurement of arterial or venous blood flow is the electromagnetic blood flow probe (EMBFP). The operation of this probe is based on the theory of electromagnetic induction in fluids. This was first described by Faraday¹⁰ in 1832, when he attempted to measure water flow with a galvanometer whose electrodes were immersed in the Thames River. Later, Fabre¹¹ suggested that it would be possible to employ this technique for the measurement of blood flow in an artery. Kolin¹² in 1936, and Wetterer¹³ in 1937, using a constant magnetic field EMBFP, were the first to successfully measure blood flow in an artery by this means. The dependence of the EMBFP's sensitivity to the encircled media was examined by Gessner¹⁴ in 1961. Shercliff¹⁵ described electromagnetic flow measurement in different media including blood. Wyatt¹⁶ derived the theoretical description of the effects of stationary and none stationary boundary fluid layers in a vessel. Bevir¹⁷ examined the conditions under which the signal from a flowmeter is independent of the velocity distribution profile and proportional to flow rate. Woodcock¹⁸ in 1976, examined the physical properties of blood and their effect on the measurement of blood flow with this technique.

Consider a vessel as shown in Fig. 1, containing a flowing conductive liquid moving through a constant magnetic field $\mathbf{B}$. If the medium (m) is moving with velocity $\mathbf{v}$, then there is a contribution to the current density in the medium, $\mathbf{j}_m$, such that,

$$\vec{j}_m = \sigma_m [\vec{E}_m + \vec{v} \times \vec{B}] \quad [2]$$

where σ_m is the conductivity of the medium¹⁹.

Fig. 1 Geometry of an electromagnetic blood flowmeter.

Since there is no net flow in the wall (w), then

$$\vec{j}_w = \sigma_w \vec{E}_w \quad [3]$$

where σ_w is the conductivity of the wall.

The induced electric potential V , used as a measure of the flow, is related to the electric field and current density such that,

$$\vec{\nabla} V_m = -\vec{E}_m = \frac{-\vec{j}_m}{\sigma_m} + \vec{v} \times \vec{B} \quad [4]$$

in the medium and,

$$\vec{\nabla} V_w = -E_w = \frac{-\vec{j}_w}{\sigma_w} \quad [5]$$

in the wall.

Applying the divergence operator to both sides of equations [4] and [5] yields

$$\begin{aligned} \vec{\nabla}^2 V_m &= \vec{\nabla} \cdot (\vec{v} \times \vec{B}) \\ \vec{\nabla}^2 V_w &= 0 \end{aligned} \quad [6]$$

In the medium, the divergence of the cross product of the velocity and the magnetic field is the divergence of the electric field, which in turn is the gradient of the potential. In contrast, the wall contains no net movement of charge carriers and hence there is no contribution from the magnetic field to the divergence of the electric field.

Referring to Fig. 1, the potentials V_m and V_w may be found by solving equation [6]. It is assumed that the velocity of the liquid has a component only in the axial direction and that no angular gradient exists. If the magnetic field is parallel to the y axis, then,

$$\text{In the medium: } \nabla^2 V_m = -B \frac{\partial v}{\partial r} \cos \varphi \quad [7]$$

$$\text{In the wall: } \nabla^2 V_w = 0$$

where φ is the angle with respect to the electrode axis as shown in Fig. 1.

The potential between the electrodes at $r=A$ and $\varphi=0,180$ can be shown (see Appendix A) to be,

$$V = 2Br\bar{V}(r)N \quad [8]$$

where the factor N involving the wall and medium properties can be expressed as,

$$N = \frac{2 \frac{\sigma_m}{\sigma_w} \frac{a}{A}}{\frac{\sigma_m}{\sigma_w} \left(1 + \frac{a^2}{A^2} \right) + \left(1 - \frac{a^2}{A^2} \right)} \quad [9]$$

The average velocity may be expressed as the volume rate of flow, Q , divided by the cross-sectional area of the tube, πa^2 . The volume rate of flow or simply the flow is the volume of medium passing through some volume per unit of time. In terms of voltage, the flow Q can be written as,

$$Q = \frac{-V\pi a}{2BN} \quad [10]$$

Equation [10] holds if the velocity of the liquid has only an axial component and is radially symmetric. For a given flow probe, the output voltage V is therefore independent of the velocity profile provided that these assumptions are satisfied. The output voltage is dependent on the volume rate of flow, a number of system parameters including the magnetic field strength and lumen diameter. It is also dependent upon the conductivity of the medium and wall materials.

2.2 Transit Time Blood Flow Probes

There are two means of measuring arterial or venous blood flow using ultrasound: a doppler shift method and a transit time measurement. The latter of these was used in this investigation. The measurement of flow by transit time ultrasound is based on the principal that the transit time of a sound wave travelling between two points is dependent on the velocity of sound with respect to the propagating medium plus the velocity of the medium in the direction of propagation of the wave. The difference in the transit times between the upstream and downstream ultrasonic pulses can be related to the flow rate of the medium. Franklin²⁰ was one of the first to use transit time ultrasound to measure flow. Franklin's probes measured the product of flow velocity and vessel diameter and were primarily employed in industrial applications. Plass²¹, in 1964, developed a flowmeter suitable for intravascular insertion and whose method of measurement employed a combination of ultrasonic pulse techniques and phase detection. Rader²² extended development of the ultrasound flow probe (ULBFP) from illumination by a point source to a line illumination perpendicular to the vessel axis and spanning the entire vessel diameter. The advantage of this method is that the signal is independent of the diameter of the vessel. Drost²³ (1978) improved Rader's technique by using a reflected acoustic pathway which largely eliminated the angle sensitivity of the flowmeter due to misalignment and non-axial flows. More sensitive versions of Drost's probes are now marketed for a variety of applications, including the validation of blood flow measurements for magnet resonance imaging²⁴ and the measurement of blood flow in test animals^{25,26,27,28,29} and humans³⁰.

Fig. 2 Geometry of an infinitesimal beam travelling from transducer 1 (2) to transducer 2 (1) passing through the vessel.

To properly characterize the behaviour of an ULBFP, it is necessary to understand the method by which flow is measured using transit time ultrasound. The time taken for an infinitesimal beam of an acoustic planewave to travel from transducer 1 to transducer 2, as seen in Fig. 2, is given by:

$$T = \int_0^L \frac{dx}{c \pm v_x} \quad [11]$$

where T is the transit time, L is the distance from transducer 1 to 2 and c is the velocity of ultrasound in the medium. The $\pm$ is for the upstream(-) and downstream(+) pulses. The velocity of blood in the vessel, v_x , is given by,

$$v_x = \begin{cases} 0 & \text{for } \bar{x} \text{ outside vessel} \\ \bar{v} & \text{for } \bar{x} \text{ inside vessel} \end{cases} \quad [12]$$

The velocity of ultrasound in whole blood or water is approximately 1500 m/s and the velocity of blood in arteries/veins is of the order 0.001 to 0.01 m/s³¹. Under these conditions, the denominator of equation [11] may be expanded for $v < c$ and terms quadratic (and higher) in v/c may be ignored. Hence the transit time can be written,

$$\begin{aligned} T &= \int_0^L \frac{dx}{c} \mp \frac{1}{c^2} \int_0^L v_x dx \\ &= \frac{L}{c} \mp T_x \end{aligned} \quad [13]$$

For a single-frequency soundwave, the transit time, T , may be expressed in terms of the phase delay in the received signal²³. For an infinitesimal beam this may be represented as,

$$dU = U_0 \sin[\omega(t-T)] dy dz \quad [14]$$

where U_0 is the transmitter to receiver signal coupling factor, and U is the unamplified signal strength.

If the two transducers are placed on the same side of the vessel and a reflector bracket is introduced to reflect the wide beam from one transducer to the other, then a similar analysis is required. Adopting the geometry of Fig. 3, a beam from transducer 1 (2) will pass through the vessel, strike the reflector on the opposite side of the vessel, pass through the vessel once more and impinge on transducer 2 (1). This is the geometry of the ULBFP used in this investigation.

The signal striking the reflector bracket is

$$U = U_0 \left(A_r \sin\left[\omega\left(t - \frac{L}{c}\right)\right] \pm wQ \frac{\omega}{c^2} \cot\theta \cos\left[\omega\left(t - \frac{L}{c}\right)\right] \right) \quad [15]$$

where A_r is the transmitter-receiver area, L is the distance from one transducer to the reflector bracket, Q is the volume rate of flow and w is the width of the transducer. Dividing out the transmitter-receiver area, the received signal can be written as,

$$U = U_0 \left(\sin\left[\omega\left(t - \frac{L}{c}\right)\right] \pm \frac{Q\omega}{c^2 h} \cot\theta \cos\left[\omega\left(t - \frac{L}{c}\right)\right] \right) \quad [16]$$

and expressing the sum as a product²³ gives,

Fig. 3 Geometry of flowmeter using a reflective acoustical pathway to illuminate the sensitivity to vessel alignment within the probe. This is the configuration of the ultrasonic flowmeter used in this investigation.

$$U = U_0 A_r \sqrt{1 + \left(\frac{Q \cot \theta}{c^2 h}\right)^2} \sin \left[\omega \left(t - \frac{L}{c} \pm \frac{\arctan \left(\frac{\omega Q \cot \theta}{c^2 h} \right)}{\omega} \right) \right] \quad [17]$$

Equation [17] is the signal received at the reflector bracket after a single pass through the vessel. The signal at the second transducer may be determined by using the same transformation which took the original signal from the first transducer to the reflecting bracket. The signal at the reflecting bracket now becomes the initial signal. After passing through the vessel for a second time, two transformations are necessary, one for the time and a second for the amplitude;

$$t \rightarrow t - \frac{L}{c} \pm \frac{\text{Arctan} \left(\frac{\omega Q \cot \alpha}{c^2 h} \right)}{\omega} \quad [18]$$

$$A_r \rightarrow A_r \sqrt{1 + \left(\frac{\omega Q \cot \alpha}{c^2 h} \right)^2}$$

Applying these transformations to equation [17], the signal at the receiver is,

$$U = U_0 A_r \sqrt{1 + 2 \left(\frac{\omega Q}{c^2 h} \right)^2 (\cot^2 \theta + \cot^2 \alpha) + \left(\frac{\omega Q}{c^2 h} \right)^4 \cot^2 \theta \cot^2 \alpha} \quad [19]$$

$$\cdot \sin \left[\omega \left(t - \frac{2L}{c} \pm \frac{1}{\omega} \left(\text{Arctan} \frac{\omega Q \cot \theta}{c^2 h} + \text{Arctan} \frac{\omega Q \cot \alpha}{c^2 h} \right) \right) \right]$$

The signal received by the transducer is both amplitude and phase modulated by the volume flow and is symmetric in the angles θ and α . The product may be expressed as a sum. Adding the upstream and downstream pulses results in a signal which is proportional to flow, to the two angles θ and α , and the velocity of ultrasound in the

medium. It also depends on characteristics of the flow probe such as the path length of the acoustic beam and the frequency of the ultrasonic crystal.

2.3 Thermal Pulse Decay Blood Flow Probe

A thermal diffusion or thermal pulse decay probe is usually constructed from an electrically resistive thermistor bead, mounted on the tip of a catheter, or in some cases, a needle. The thermistor is encased in a glass bead and surrounded by a thin layer of epoxy. Power is electrically deposited in the thermistor and is subsequently lost to the surrounding tissues in the form of heat. The rate of heat dissipation can be related to the flow rate in the tissue.

In 1933 Gibbs³² described the simple construction of a thermoelectric blood flow recorder which was in the form of a thermocouple bead at the tip of a needle. The operation of a thermal pulse decay probe or thermal diffusion probe was described by Goldenberg³³ in 1952. Chen³⁴ (1981) improved Goldenberg's method by using small thermistor probes with the assumption that the probe was a point source of heat and the model he derived is called the point source model (PSM). The validity of the PSM was further justified by Arkin³⁵, who analyzed the effects of bead radius, inhomogeneities in the surrounding tissues and the trauma associated with probe placement. Building on the PSM, Diedrich³⁶ in 1988, developed the spherical source model (SSM), to take into account the finite bead thickness. Thermal dilution probes have been constructed which are capable of measuring the thermal conductivity and diffusivity of tissues, as well as, the local perfusion around the probe^{37,38,39,40}. In the sections to follow, only the point source model for the thermal pulse decay probe will be examined. The experiments performed on the thermal pulse decay probes will be discussed, the results shown and the significance of the data will be examined.

The derivation of the thermal pulse decay method for the PSM assume that the bio-heat transfer equation (BHTE) describes the thermal interactions in the surrounding media.

The bio-heat transfer equation, as described by Pennes⁴¹, is only an approximate representation of the heat transfer in tissue. However, since the BHTE is solvable, most thermal techniques for determining tissue properties use the BHTE to obtain their solutions³⁶.

Other investigators^{42,43} have formulated equations which have a more robust physical basis than the BHTE, but these are more difficult to solve. Even though it is a limited formulation, the BHTE has been useful for predicting temperature fields, including those in hyperthermia^{44,45}.

The bio-heat transfer equation is a general differential equation which describes the heat transfer in tissue. For a point source of heat, the one dimensional BHTE can be expressed as³⁶:

$$\rho_t c_t \frac{\partial \Theta_t}{\partial t} = k_t \nabla^2 \Theta_t - W c_b \Theta_t + P_b \theta(t) \theta(t-\tau') \quad [20]$$

where Θ_t is the temperature above ambient. The thermal conductivity of the tissue, k_t , the specific heat, c_t and tissue density, ρ_t are assumed constant as is the specific heat of blood, c_b . The density of blood and tissue are equal. The specific heats for tissue and blood are also assumed to be equal. The power supplied by the thermistor bead is included in the heat source term $P_b \theta(t) \theta(t-\tau')$, where τ' is the heating time. The theta functions restrict the source term P to be between 0 and τ' . The effect of perfusion, W , is contained in the $W c_b \Theta_t$ term. Note that the metabolic heating term, usually written as Q_m , has been neglected since it is assumed that it does not significantly contribute to perturbations in temperature over short times⁴⁶. Equation [20] may be rewritten in the form,

$$\frac{\partial \Theta}{\partial t} - a^2 \nabla^2 \Theta + b \Theta = f(x, t) \quad [21]$$

where $a^2 = k_t / \rho_t c_t$, $b = W / \rho_t$ and $f(x, t)$ is the power density deposited into the sphere, $P \delta(t) \delta(t - \tau')$ divided by $\rho_t c_t$.

The most general form of equation [21] is for a distributed (finite size) heat source with a heterogeneous composition, which is embedded in a nonhomogenous tissue bed having various thermal properties. The point source model assumes that the probe or power source is infinitely small and is surrounded by an infinite, homogenous tissue region. The assumption of tissue homogeneity is valid, if the tissue is homogenous over a volume which is greater than the effective measurement volume sensed by the thermal probe, equivalent to approximately four times the probe radius³⁵. The solution of equation [20] for the point source model can be determined by means of Green's function analysis³⁶. The Green's function, $G(x, \xi; t, \tau)$, to be found must solve the nonhomogeneous partial differential equation expressed in equation [21] and must satisfy,

$$\frac{\partial G}{\partial t} - a^2 \frac{\partial^2 G}{\partial x^2} + bG = \delta(x - \xi) \delta(t - \tau) \quad [22]$$

An expression for G may be found by transform methods⁴⁷. The fourier transform with respect to x of equation [22] gives,

$$\frac{dG_F}{dt} + a^2 k^2 G_F + bG_F = \frac{e^{ik\xi}}{\sqrt{2\pi}} \delta(t - \tau) \quad [23]$$

where $G_F = \mathcal{F}\{G\}$ and k is associated with the transform and not to be confused with the tissue conductivity, k_t .

Taking the Laplace transform of equation [23] with respect to t ,

$$sG_{FL} + a^2k^2G_{FL} + bG_{FL} = \frac{e^{ik\xi}}{\sqrt{2\pi}}e^{-s\tau} \quad [24]$$

where $G_{FL} = \mathcal{L}\{G_F\}$. From equation [24], G_{FL} may be found and the inverse Laplace transform used to solve for G_F . Using the shifting and attenuation properties of Laplace transforms⁴⁶,

$$G_F = \frac{e^{ik\xi}}{\sqrt{2\pi}}e^{-a^2k^2(t-\tau)}e^{-b(t-\tau)}\theta(t-\tau) \quad [25]$$

where $\theta(t-\tau)$ is the step-function. Using the convolution theorem, the inversion of equation [25] with respect to the Fourier transform gives,

$$\begin{aligned} G &= \theta(t-\tau)e^{-b(t-\tau)} \int_{-\infty}^{+\infty} \theta(x'-\xi) \frac{1}{\sqrt{4\pi a^2(t-\tau)}} e^{-(x-x')^2/4a^2(t-\tau)} dx' \\ &= \frac{\theta(t-\tau)e^{-b(t-\tau)}}{\sqrt{4\pi a^2(t-\tau)}} e^{-(x-\xi)^2/4a^2(t-\tau)} \end{aligned} \quad [26]$$

The solution to the nonhomogeneous partial differential equation may be expressed as,

$$\Theta(x,t) = \int_0^{+\infty} \int_{-\infty}^{+\infty} G(x|\xi;\tau) f(\xi,\tau) d\xi d\tau \quad [27]$$

Substitution of equation [26] into equation [27] gives,

$$\Theta(x,t) = \int_0^{+\infty} \int_{-\infty}^{+\infty} \frac{\theta(t-\tau)e^{-b(t-\tau)}}{\sqrt{4\pi a^2(t-\tau)}} e^{-(x-\xi)^2/4a^2(t-\tau)} f(\xi,\tau) d\xi d\tau \quad [28]$$

Substituting the function $f(\xi, \tau) = P\theta(\tau)\theta(\tau - \tau')/\rho_i c_i$ makes the integral over ξ trivial and hence, Θ_i may be written as,

$$\Theta(x, t) = \frac{P}{\rho_i c_i} \int_0^{\tau'} \frac{e^{-\frac{W}{\rho_i}(t-\tau)}}{\sqrt{4\pi \frac{k_i}{\rho_i c_i}(t-\tau)}} e^{-(\rho_i c_i x^2 / 4k_i)(t-\tau)} d\tau \quad [29]$$

This is the solution to the one-dimensional problem. The solution to the three-dimensional problem may be found by symmetry, since there will be, in addition to the exponential term involving x^2 , exponential terms involving y^2 and z^2 . This will allow for the substitution of $r^2 = (x^2 + y^2 + z^2)$. There will also exist two more terms involving $(t - \tau)^{1/2}$, one for y and one for z . The solution to the three-dimensional problem is therefore,

$$\Theta(r, t) = \frac{P(\rho_i c_i)^{\frac{1}{2}}}{8(\pi k_i)^{\frac{3}{2}}} \int_0^{\tau'} (t - \tau)^{-\frac{3}{2}} e^{-\frac{W}{\rho_i}(t-\tau)} e^{-r^2/(4k_i/\rho_i c_i)(t-\tau)} d\tau \quad [30]$$

This is the solution found by Arkin⁴⁸ et al. for the temperature field due to a constant power pulse point source in a infinite, homogenous, perfused media.

The integral is further simplified since, the point of measurement is at $r=0$. The exponential term involving the radial component of the temperature distribution may be neglected. The temperature as a function of time at $r=0$ can be written as,

$$\Theta(0, t) = \frac{P(\rho_i c_i)^{\frac{1}{2}}}{8(\pi k_i)^{\frac{3}{2}}} \int_0^{\tau'} (t - \tau)^{-\frac{3}{2}} e^{-\frac{W}{\rho_i}(t-\tau)} d\tau \quad [31]$$

Fig. 4 The theoretical change in temperature of the thermistor bead for various perfusions. The medium is water at 39.0°C. The heating time is 1.667 s and the deposited power is 33 W/cm³.

To determine the change in temperature, $\Theta(0,t)$, it is necessary to evaluate the remaining integral in equation [31]. The solution to may be expressed as,

$$\Theta(0,t) = \frac{P(\rho c_p)^{1/2}}{4(\pi k_p)^{3/2}} \left(\frac{1}{e^{\frac{W_b(t-\tau')}{\rho_i}} \sqrt{t-\tau'}} - \frac{1}{e^{\frac{W_b t}{\rho_i}} \sqrt{t}} \right) + \sqrt{\pi \frac{W_b}{\rho_i}} \left[\operatorname{erf} \sqrt{\frac{W_b(t-\tau')}{\rho_i}} - \operatorname{erf} \sqrt{\frac{W_b t}{\rho_i}} \right] \quad [32]$$

where erf is the error function. For a given value of perfusion, the temperature changes as a function of time may be determined, (see Fig. 4). It may be noted that as perfusion increases, the maximum temperature achieved decreases, reaching the value of zero for infinite perfusion. For zero perfusion, equation [32] is greatly simplified and may be written as,

$$\Theta(0,t) = \frac{P(\rho c_p)^{1/2}}{4(\pi k_p)^{3/2}} \left(\frac{1}{\sqrt{t-\tau'}} - \frac{1}{\sqrt{t}} \right) \quad [33]$$

For $t \gg \tau'$, the temperature change in both equations [32] and [33] approaches zero, implying that the bead temperature approaches the thermal background temperature a long time after the heating pulse is turned off.

Using equation [32], the temperature increase as a function of time may be plotted for low, Fig. 5, and high, Fig. 6, perfusion rates. Notice that for low perfusion there is little change in the temperature time curve from the zero perfusion curve. The material properties and parameters used for these plots are given in Table I. The same properties are shown for water and saline as a comparison.

Fig. 5 The theoretical temperature above background due to the heating pulse is shown for perfusion rates typical of kidneys.

Fig. 6 The theoretical temperature rise due to the heating pulse as a function of time is shown for high rates of perfusion.

Parameter	Water	Saline	Kidney
ρ (g/cm ³)	1	1.01	1
c (J/g°C)	4.18	4.13	3.448
k (mW/cm°C)	6.25	6.20	10
τ' (s)	1.667	1.667	1.667
P (W/cm ³)	33	33	33

Table I The constants used for the theoretical plots of the temperature increase of the thermistor due to the heating pulse in water, saline and kidney.

The operation of the thermal pulse decay probe was developed from the bioheat transfer equation for a point source of heat and the temperature increase of the temperature sensor was determined. The temperature increase, given by equation [32], is a function of the flow or perfusion W around the thermal bead, the thermal conductivity, specific heat and density of the tissue as well as the deposited power. The probe may therefore be characterized by varying the flow rate in the vicinity of the probe and type of medium. The data from these experiments may then be used to assess the in vivo performance of the probe. The experimental details, in addition to the method by which these parameters were varied and how they affected the probes, is discussed below.

2.4 Radioactive Tracer Microsphere Technique

The radioactive tracer microsphere (RTM) technique is a well established method of measuring in vivo blood flow and is often considered to be the gold standard. The technique involves the injection of RTMs into an animals blood supply (usually the arterial side). The flow is determined by the number of trapped microspheres in the tissue capillary bed. The RTM technique evolved from the measurement of blood flow with diffusible indicators, first described by Stewart⁴⁹. To quantify cardiac output and regional blood flow in dogs he used the electrical conductivity of a sodium solution as an indicator. Subsequently, Hamilton⁵⁰, working with dyes, further developed the indicator technique, examined its principles and is recognized as popularizing the technique in human and animal subjects. Rudolph and Heymann⁵¹ are credited with developing the RTM technique, in which they determined flow to an organ by forming the ratio of the organ's radioactivity to the total body radioactivity and multiplying by the cardiac output. This approach to RTM is awkward if the flow to only one organ is required, since every organ must be counted and the cardiac output measured by some independent method⁵².

In this work a different methodology is adopted. A suspension of microspheres is injected into the left ventricle over a 10-20 second period. Blood is sampled at a known

rate from an artery for a period of time longer than the transit time of the microspheres to any organ. This sample acts as a reference pseudo-organ (R). The specific organ flow rate is calculated from the product of the flow rate to the reference with the ratio of organ to reference activity. The cardiac output may be determined by replacing the organ activity by the total activity of the injected microspheres⁵³. Today, the RTM technique is considered to be the primary standard in terms of measuring regional and systemic blood flow in experimental animals^{54,55,56}.

If an amount of indicator is injected into the arterial blood stream, then the amount da of the indicator trapped in some tissue sample in some time dt will be the product of the flow rate $q_s(t)$ to that tissue sample, the concentration $c_a(t)$ of indicator in the arterial blood and the trapping efficiency ϵ such that,

$$da = q_s(t)c_a(t)\epsilon dt \quad [34]$$

Trapping efficiency is defined as the percentage of microspheres which are captured by the tissue on the first circulation through the organ and are not subsequently released.

Assuming that the trapping efficiency is 100%, then the total amount of indicator trapped in the tissue sample will be

$$A_s = \int_0^T q_s(t)c_a(t)dt \quad [35]$$

where T equals the injection time plus the transit time of the indicator to the tissue sample. The number of microspheres in this reference organ is similarly given by

$$A_R = \int_0^T q_R(t) c_a(t) dt \quad [36]$$

where $q_R(t)$ is the rate at which blood is sampled.

The ratio of the organ activity to the reference activity is,

$$\frac{A_S}{A_R} = \frac{\int_0^T q_S(t) c_a(t) dt}{\int_0^T q_R(t) c_a(t) dt} \quad [37]$$

Immediately after injection, the microspheres are assumed to be well mixed with the blood such that the concentration of microspheres in the artery may be assumed to be a constant during the pulse at all points in the arterial supply. Then, equation [37] can be written as,

$$\frac{A_S}{A_R} = \frac{\int_0^T q_S(t) dt}{\int_0^T q_R(t) dt} \quad [38]$$

The average flow is defined as,

$$\bar{Q} = \frac{1}{T} \int_0^T q(t) dt \quad [39]$$

substituting equation [39] into equation [38] yields,

$$\frac{A_s}{A_R} = \frac{\bar{Q}_s}{\bar{Q}_R} \quad [40]$$

and hence the flow to the sample is given as,

$$\bar{Q}_s = A_s \left[\frac{\bar{Q}_R}{A_R} \right] \quad [41]$$

If the activity of the reference organ is counted and the withdrawal rate is known, then by counting the activity of the sample, or section of the sample, the flow rate to the sample or section is determined.

The assumptions required in the above analysis were:

- 1) that the microspheres are well mixed within the blood stream and are uniformly distributed throughout the body according to the distribution of blood flow.;
- 2) The microspheres have rheologic properties similar to elements in the blood and be distributed throughout the tissue in the same manner as whole blood.;
- 3) The introduction of the microspheres into the body, specifically the organ under investigation and the heart, should not result in significant changes in the circulation.;
- 4) Microspheres should remain trapped in the tissue.;
- 5) The trapping efficiency ϵ is 100%⁵³.

Since a trapping efficiency of 100% for the organ under study is a crucial assumption of the model, it is necessary to further examine this assumption. The trapping efficiency of the kidney for the 15 μm microspheres used in this investigation has been studied by numerous investigators^{52,57,58,59}. As blood passes through the organ it must eventually traverse the capillary bed. The exception here is where there are arterial or venous shunts. The average capillary diameter is approximately 6 μm and the nominal diameter

of the microspheres used is $15 \pm 3 \mu\text{m}$. The microspheres are therefore large enough to become embedded in the capillary bed, yet small enough such that larger vessels are not blocked, preventing portions of the circulation system from becoming obstructed. It may be noted that the diameter of red blood cells (6 to $10 \mu\text{m}$) is also larger than the average capillary diameter, however, unlike microspheres, the cells deform as they pass through the capillary bed. It is interesting to note that there are some diseases, such as sickle cell anaemia, in which the red blood cell membrane is too rigid and their passage through the microvasculature is impaired. In addition, microspheres of this small diameter are preferred over larger diameter spheres, since they better approximate the actual distribution of blood flow⁶⁰.

One very important assumption is that only a small percentage of the microvasculature of the kidney is occluded by the microspheres such that there is no significant alteration in the circulation system. This assumption may be validated in the following manner. The kidney is a highly vascular organ, with a capillary density⁶¹ of $10^5/\text{cm}^3$. The average kidney has a volume of approximately 100 cm^3 and therefore contains approximately 10^7 capillaries. The number of microspheres delivered to the organ has been determined to be 2.8×10^5 , (see section 3.4). If one microsphere entirely blocks one capillary, then only 3% of the kidney will become obstructed for each microsphere injection used. Single microsphere injections to the kidney should therefore not significantly alter renal function. Repeated injections may cause a decrease in the total flow through the organ, for example, 6 injections would reduce the flow by 18%. Note that this is a worse case scenario, it is more likely that several microspheres would block a single capillary, effectively reducing this blocking effect.

3. Materials and Methods

In this work a series of experiments have been designed to compare and contrast four methods of measuring blood flow: square wave electromagnetic, transit time ultrasound, a thermal pulse decay technique and the radioactive tracer microsphere method. The designs of each of these techniques will be discussed in turn as will be the types of experiments performed.

3.1 Electromagnetic Flowmeters

The electromagnetic blood flowmeter EMBFM consists of two principal components: the transducer and the meter. There are three types of EMBFM. They differ principally in the magnetic field which is applied across the vessel. These are the DC flowmeter, the sine-wave AC flowmeter and the square-wave flowmeter, the latter being the device used in this investigation. The square-wave EMBFM is powered by a high resistance square-wave source such that the generated field is effectively a DC field with periodic reversing polarity. The theoretical analysis of 2.1 is valid since the magnetic field is assumed to be constant during the measurement part of the cycle.

Two designs of transducer or flow probe were used with the square wave EMBFM in this investigation; the perivascular and the cannulating probes. A perivascular EMBFP is one in which the probe is placed around the vessel. This type of flow probe requires that the vessel be stripped down such that no fat or muscle remain, although no trans-section of the vessel is required. Perivascular probes are routinely used in vivo for acute applications and chronic implants to measure blood flow around arteries or veins. A cannulating EMBFP is rigid tube with connections on either end. To use this probe requires ligation of the vessel and suturing the ends of the vessel to the probe connectors. These are normally used in extracorporeal applications, such as flow studies using isolated perfused organs⁶². In this investigation, both a 4 mm perivascular⁶³ and a 4 mm i.d. cannulating⁶⁴ EMBFP were used.

3.2 Transit Time Flow Probes

Two types of transit time ULBFs were used in this investigation, the perivascular and cannulating versions. Both probes consist of two ultrasonic transducers and a fixed acoustic bracket to reflect the beams. Both transducers are positioned on the same side of the probe body and the reflecting bracket is located midway between and opposite the two transducers. The cannulating transit time ULBF has a clear plastic housing with stainless steel inlet and outlet connections. The clear housing permits detection of air in the sensing chamber of the probe. The transit time probe, unlike the electromagnetic probe, does not require a tight fit around the vessel. However, saline or a jell must be used between the vessel and the probe to ensure proper acoustic coupling. Perivascular probes measure blood flow in arteries and veins and are used in vivo for chronic and acute experiments.

Both types of ULBF are available in a variety of diameters. In this study, 4 mm and 8 mm diameter perivascular (Transonic #4RB and #8RB) and a 4 mm cannulating ULBF (Transonic #4N), with stainless steel fittings⁶⁵ were used.

Flow through the perivascular and cannulating probes was monitored with a transit time ultrasound blood flow meter, (Transonic #T101⁶⁶). The flow meter identifies which probe is connected to it, its size and calibration factor and adjusts the meter's flow ranges and gains accordingly. The flow meter has circuitry to detect and display low signal strength and malfunctions in the flow sensors.

3.3 Thermal Pulse Decay Probe

The thermal pulse decay probe used in this investigation consists of a thermistor bead which is one arm of a Wheatstone bridge⁶⁷, as shown in Fig. 7. The bridge circuit is composed of four arms, three of which are precision resistors. The remaining arm is composed of the thermistor in parallel with a high voltage source which provides the heating current. This heating current is controlled by an optoswitch (4N26 8117).

Fig. 7 The thermal pulse decay probe consists of a Wheatstone bridge in which one of the arms is a thermistor. The circuit, data acquisition system and computer components are shown.

Fig. 8 A cross section of the thermal pulse decay probe showing the construction materials and component sizes.

The thermistor, mounted to the tip of a #20 thin catheter and held in place with a thin layer of clear epoxy⁶⁸, is attached to the bridge by long thin (64 micron diameter) leads as illustrated in Fig. 8. A total of four probes of this design were built. A small (2-3 mm diameter) thermistor was chosen, since the probe must be small enough to approximate a point source and must not have a large thermal mass. In addition, the probe should be small to minimize the trauma to the surrounding tissue. Since the goal is that the probes eventually be used in humans, the small size of the probe facilitates the introduction of the probe into tissue and minimizes patient discomfort. This probe could be used for either perfusion (tissue) or bulk flow measurements.

In order to study bulk flow measurements, the thermistor was placed in an 8 mm diameter T type connector and used as a cannulating probe. This size (8 mm) of the cannulating tube was used since the theoretical effective volume of measurement of the probe is approximately 4 probe radii⁴⁶. Since the probes vary in radius from 1 to 2 mm, the probe housing should not exceed 8 mm. In this manner, the TPD probe should be measuring the total flow rate passing through the T connector.

The thermistor is heated resistively by a heating current passing through it. When the heating current (16.2 ± 0.1 mA) is removed the temperature of the thermistor decays to the surrounding background temperature. The voltage across the bridge is a function of the resistance in the arms. The resistance of the thermistor exponentially decreases with temperature over the range -40 to 100°C, but is assumed linear, (see Fig. 9), for the range of temperatures considered here, 35 to 50°C. The resistance of the thermistor bead over this limited range can be expressed as,

$$R_{Therm} = K_1 - K_2[T_{REF} + \Theta(0,t)] \quad [42]$$

where $K_1 = 2964.075 \, \Omega$, $K_2 = -43.709 \, \Omega/^\circ\text{C}$, T_{REF} is the reference or ambient temperature and $\Theta(0,t)$ is given by equation [32].

Fig. 9 The resistance of the thermistor bead is shown as a function of temperature over the range of interest, suggesting a linear relationship.

The voltage across the bridge, V_s , may be written in terms of the applied voltage V_a and the resistance of the various arms,

$$V_s = V_a \left(\frac{R1}{R1 + R2} - \frac{R_{Therm}}{R_{Therm} + R3} \right) \quad [43]$$

Using equation [43], the voltage across the bridge may be determined theoretically for different perfusion rates. Using the data for water presented in Table I above and the appropriate resistor values given in Fig. 7, the voltage across the bridge as a function of time is shown in Fig. 10 for low and high perfusion rates respectively.

In principle it would be possible to fit the measured voltage data with equation [43] and subsequently determine the perfusion term, but in practice the function is too complex and difficult to fit with any accuracy in its present form. Several researchers expand the function in a power series, but this is often impractical since the series regularly requires twelve terms in order to converge⁴⁸.

It was postulated that the theoretical voltage decay might be characterized by a single exponential function ($y=A\exp[Bt]+C$) and that the decay constant of this exponential would act as a measure of perfusion. To support this assumption, a typical theoretical voltage curve is shown in Fig. 11 and overlying the curve is the single exponential fit to the data. The decay constant for this data was plotted for different perfusions and is shown in Fig. 12. The decay constant determined by the exponential parameterization of the theoretical data decrease with increasing flow rate and a second order polynomial may be used to approximate the data revealing a slight upward trend in the data for high perfusions. This suggests that by measuring the voltage decay due to the heating pulse and fitting the data with a single exponential function of the form $y=A\exp[Bt]+C$, the decay constant B will be an indication of the perfusion.

Fig. 10 The theoretical voltage across the bridge circuit as a function of time, for high perfusion rates in water.

Fig. 11 The theoretical voltage decay as a function of time for low and high perfusions are fit with a single decaying exponential function shown as a bold solid line.

Fig. 12 The decay constants from the exponentially fitted theoretical data are plotted against the perfusion. A second order polynomial is fit to the data.

The heating pulse (9.09 V), switch voltage (1.25 V) and bridge voltage (0.65 V) are applied and measured with a Keithly Series 500 data acquisition system. A program called THERMIST was written in ASYST version 3.10, a high level programming language. ASYST was also used to determine the fit parameters of the measured exponential voltage decay curves. The program listing is in Appendix C. The computer, data acquisition system and the TPD probe are illustrated in Fig. 7.

The voltage-time decay data from the resistor bridge were parameterized with exponential functions of the form $V = A \exp[Bt] + C$. In Fig. 13, a sample set of data from Probe #1 shows the decay of the bridge voltage over time after a 1.667 s heating pulse. The decay constant of the probe B, used as an indication of the flow around the probe, was determined for a range of flows, type of media, including sand and sponge and for a range of background temperatures.

The functional form of the temperature increase, equation [32], due to the heating pulse suggests a number of experiments which might be preformed. This formula implies that the measured temperature and hence the measured voltage, equation [43], is a function of not only the flow, but the thermal conductivity, density and specific heat of the medium. To quantify the probe's response to changes in these quantities the thermal probe was calibrated against flow in several media including sponge and sand, as well as, a number of biological tissues. The probes were also calibrated against the flow rate of saline and water for temperatures in the range 35 to 45°C. All of these experiments are described below. In addition to the parameters mentioned, the voltage measured across the bridge is also a function of the deposited power density and the heating time, but these were constant in all of the experiments, ($P = 33 \text{ W/cm}^2$ and $\tau = 1.667 \text{ s}$).

The plots of the decay constant from the data under various conditions constitute the calibration curves for the thermal probes. In this manner the TPD probes were calibrated for various parameters such as background temperature, medium type and different kinds of porous media such as sponge, sand, kidney and other tissue. By calibrating the probe

Fig. 13 A heating pulse is applied to the TPD probe (#1) and the voltage decay of the resistor bridge network is parameterized with the function $y = A \exp[Bt] + C$. The fits to the data lie coincident on the curves.

for a number of situations, the response of the probe in vivo may be better understood and changes to the signal interpreted. To this end, an isolated saline perfused swine kidney was used to calibrate the TPD probe in vivo using the radioactive tracer microsphere method to determine the local perfusion around the probe.

3.4 RTM Method

To examine the RTM technique under in vitro and in situ conditions it was necessary to use isolated and native swine kidneys. Three female Yorkshire pigs weighing between 30-40 kg were premedicated and prepared as described in Appendix D.

There are two basic pig experiments performed in conjunction with the RTM method, specifically, the in situ and the externalized swine kidney. The purpose of the in situ experiments is three fold;

- 1) to verify the assumption that the microspheres are well mixed with the blood.
- 2) to determine if the injection of microspheres result in significant changes in the circulation.
- 3) to measure the trapping efficiency of the kidney, using an autoperfused kidney. An autoperfused kidney is one which has been removed and is then supplied with blood from another artery, such as the femoral.

The isolated saline perfused swine kidney experiments have one specific purpose, to calibrate the RTM technique's measurement of total flow rate against the pulsatile rotary pump.

Tracer microspheres are polystyrene beads containing a small amount of radioactive material. By annealing at over 400°C, the radioactive material becomes sealed within the sphere and no isotope leaching occurs. The radioactive tracer microspheres (Dupont Nentrac) used in this investigation were (15±3) µm diameter beads labelled with one of six isotopes; ⁵⁷Co, ⁹⁵Nb, ¹⁰³Ru, ¹¹³Sn, ⁴⁶Sc and ⁸⁵Sr. The mean peak energy and half

lives of these isotopes are shown in Table II. As stated in section 2.4, this size of microsphere is considered to be ideal for the study of renal haemodynamics.

The microspheres arrive from the manufacturer in 370 MBq/ml samples. This sample is diluted with saline to a concentration of 0.185 MBq/ml. Microsphere samples containing a total of 1.85 MBq were prepared by adding 0.02% Tween-saline solution to a total volume of 10 ml. Tween (polyoxyethylene-80-sorbitan mono-oleate) acts as a surfactant to prevent microsphere aggregation. There are 4.5×10^8 microspheres/ml in the stock solution, and hence there are approximately 2.25×10^6 microspheres in the 10 ml solution. Since each kidney receives an eighth of the cardiac output, there should be 2.8×10^5 microspheres distributed in each kidney.

The solution is warmed to room temperature and either vortexed or sonicated for five minutes prior to injection. Immediately after vortexing, the 10 ml solution containing 2.25×10^6 microspheres is transferred to a 30 ml syringe using a long stainless steel needle. With the animal stable, the microsphere solution is injected in the left ventricle via a #7 French pig tail catheter at a rate of 1 ml/s. The line is subsequently flushed with 10 ml of saline at the same rate. It has been shown that microsphere injections into the left ventricle and withdrawal of the reference sample from the arterial side is an appropriate method for pigs⁶⁹. The reference sample, or pseudo organ, was withdrawn at a rate of 24.7 ml/min, using a Harvard syringe pump and a 30 ml syringe. This sample was immediately transferred to a 50 ml conical test tube.

Before the counting procedure is described it is useful to discuss the number of microspheres which are trapped in the kidney with reference to the number required to

Element	Half Life ($T_{1/2}$) (Days)	Mean Energy (E_γ) (keV)
^{57}Co	271	122.06 136.471
^{46}Sc	83.85	889.26 1120.52
^{113}Sn	115.2	255.12 391.69
^{85}Sr	64.5	513.99
^{103}Ru	39.4	497.10
^{95}Nb	35.1	765.79

Table II The isotopes used in this investigation are shown with the half life and mean peak energies of the gamma rays for each isotope. achieve proper counting statistics.

It was previously determined that each kidney receives approximately 2.8×10^5 microspheres. If a kidney is dissected into 20 equal sections, and there is a uniform distribution of spheres throughout the organ, then there will be approximately 1.4×10^4 microspheres in each sample. This is considered to be more than adequate in terms of counting statistics, which require a minimum of 400 microspheres/sample^{60,70}.

To prepare the kidney for counting, the sample was separated into ventral and dorsal halves. Each half was sectioned into 1.5 cm wide samples from the superior to inferior side of the kidney and placed in a 50 ml conical polyethylene test tube. The reference blood samples, originally collected in 50 ml conical test tubes, required no manipulation prior to counting. The activity of the blood remaining in the reference syringes was considered to be negligible and was not counted.

The activity of the blood and tissue samples were counted in a 2048 channel Ge(Li) liquid nitrogen cooled gamma ray spectrometer. The detector to source distance was 3 cm. The detector was calibrated with 5 sources with known energies in the range 0-1300 keV. The resulting calibration was used to determine which peaks in the spectrum of a sample arose from a given isotope. Samples were placed in the detector system and counted for 10 minutes. An acquisition time of 10 minutes was chosen since it provided sufficient counts/channel to be analyzed and allowed all the samples, as many as 20 per kidney, to be counted in approximately 3.5 hours. Since all the samples from a given experiment including the references are counted in a relatively short period of time, no correction for radioactive decay of the sample was required. The spectrum of a sample was analyzed with a program called D40 written by the National Research Council (NRC) of Canada. The program determined the energy of a given peak, as well as the area under the peak, or the activity. No corrections for detector efficiency are required since the RTM technique relies on the relative as opposed to absolute activities of the samples. No correction was made for the extended source size of the samples, but it may be noted that all the tissue samples had approximately the same volume.

Five sources were used to periodically calibrate the detector. A typical detector energy calibration curve is shown in Fig. 14. The linear fit to the data is $y=0.572x+2.00$, $r=.999$. After calibrating the detector with standard sources, the tissue or reference sample is placed in the detector. The calibration curve is used to determine the energies of the peaks in the samples. Fig. 15 shows the partial energy spectrum of a source containing ^{57}Co . The two main peaks at 122 and 136 keV are clearly visible above the background.

To determine the typical error on the total count for a sample, a sample was counted repeatedly and the standard deviation of the data used. The count error for an entire kidney was 8%. In this investigation a conservative 10% error will be used for the count from the sample. The error in the counts of the reference and flow (24.7 ± 0.5) ml/min to the reference are negligible in comparison to the error of the sample and are therefore not included in the estimate of the flow to the sample.

Fig. 14 Energy Calibration of Ge(Li) detector using 5 sources of known energies in the range 0 to 1300 keV.

Fig. 15 Typical energy spectrum of a reference blood sample. The main peaks are due to ^{57}Co .

3.5 Description of Experiments

It has been established that the accurate measurement of blood flow or perfusion is necessary. The problem may be stated; how is the accuracy of a given probe determined? A review of the literature reveals that, while some researchers calibrate one method of blood flow measurement against a second method^{71,72,73}, many researchers do not calibrate their devices against known flows, either in vivo or in vitro^{74,75,76}. If a probe is not thoroughly calibrated then details like the zero offset and effect of temperature are unknown. The probe or technique may be inaccurately reporting flow. In this work all of the devices are calibrated against known flows for a variety of situations. To simplify the description of the in excess of fifty different types of experiments, four flow charts are presented. The first chart, shown in Fig. 16, depicts the relationship of the in vivo and in vitro experiments. The next three charts deal with the experiments for the electromagnetic, ultrasonic and thermal pulse decay probes in vitro shown in Fig. 17, Fig. 18 and Fig. 19 respectively.

The basic experimental setup used to calibrate the electromagnetic, ultrasonic and TPD probes in vitro is shown in Fig. 20. Fluid from the pump or rotameter passes through a heat exchanger, which in conjunction with the heated reservoir maintains the medium temperature to within 0.5°C of the desired test temperature. Temperature was monitored at all times with multiple type K thermocouples⁷⁷ inserted in the reservoir and in proximity to the probe. After passing through the probe, the medium is returned to the reservoir. All tubing connections are made of 6411-17 Masterflex tubing. The voltage signal is recorded on a Brush 220 stripchart recorder set at 50 mV/div and varying tape speeds. Since the pump and rotameter had been precalibrated, the flow through the vessel is known. Alterations to this basic setup are discussed below.

Flow Chart of Experiments

Fig. 16 Flow chart of all experiments, both in vivo and in vitro.

Electromagnetic Flow Probe Experiments

Fig. 17 The in vitro experiments performed with the cannulating and perivascular electromagnetic flow probes.

Thermal Pulse Decay Probe In vitro

Fig. 19 The flow chart of the in vitro experiments performed using the cannulating TPD probe and the flow cell.

Fig. 20 Experimental configuration used to calibrate the various flow probes.

3.5.1 Type of Flow

There are essentially two types of fluids: Newtonian and Non-Newtonian. Newtonian fluids are fluids of whose viscosity does not depend on velocity⁷⁸. Examples of newtonian fluids are water and saline. Blood, on the other hand, is a Non-Newtonian fluid. A review of the types of fluids and flows may be found in Tritton⁷⁸. Both types of fluid may have steady state or pulsatile velocity profiles. Two types of flow were studied: steady and pulsatile. Steady flow was provided by gravity feed from a constant temperature bath and controlled with a precalibrated rotameter⁷⁹. Pulsatile flow was provided by a precalibrated, digital, rotary pump⁸⁰. To calibrate the probes, the flowmeter was set to a given flow rate between 0 and 300 ml/min. The temperature and the output signal of the probe were recorded.

3.5.2 Medium Temperature

The temperature of the solution in the reservoir was adjusted to the desired temperature and monitored by type K thermocouples. A heat exchanger, supplied by the same reservoir was used to ensure that the medium in the flow circuit was maintained at the desired temperature. A thermocouple positioned in close proximity to the vessel/probe assembly was used to ensure that the temperature of the measurement point was within 0.5°C of the reservoir temperature. In addition, short lines were used to minimize heat loss. The experiment was repeated for several temperatures in the range 20-50°C.

3.5.3 Different Media

Three media were investigated: saline, a mixture of red blood cells and isotonic saline (195 ml of red cells + 1121.5 ml isotonic saline) and whole blood were used as perfusate. Blood and the blood-saline solution are too viscous to be used in conjunction with the rotameter, and hence only pulsatile flows could be studied with these media. The blood-saline was heated in a beaker using a heater stirrer bath to avoid contaminating the reservoir. The temperature of the media was maintained with a heat exchanger, and monitored in the manner described above.

To calibrate the probes with whole blood a single female swine was surgically prepared as per Appendix D. A 5 cm section of the left femoral artery was isolated and a cannula inserted at the inferior side. The cannula was attached to the input of the pulsatile pump and the outflow returned via the left femoral vein. The cannulating ultrasonic and electromagnetic probes were placed between the outflow of the pump and the femoral vein. After removing all fat tissue, the 4 mm perivascular probe was placed on the femoral artery. Care was taken to ensure that the cannula was not in the sensing element of the probe. Saline was used as a couplant material. Outflow from the artery, controlled by the calibrated rotary pump, passed through the probe and was returned to the animal via the left femoral vein.

3.5.4 Vessel Type, Orientation and Position

It is often necessary to repeat measurements for a given setup, both in vivo and in vitro. Since it is impossible to exactly reproduce vessel orientation on a day-to-day basis, or even to ensure zero movement during an experiment (particularly in vivo), it is important to quantify the effect of vessel orientation and position on the measurement of flow. In addition, it is often necessary to compare flow through different types of vessel (tubing, aorta). Several types of tubing were tested; these include latex tubing (4 mm o.d.), Masterflex 6411-17,14,15,16, Tygon, Spectrum dialysis tubing with both 2.5 mm i.d. and 6.8 mm i.d., as well as sections a unfixed swine aorta obtained at death.

The signal received from the ultrasonic probe has been shown (section 2.2) to depend on the orientation of the vessel within the probe. The reflector bracket design is an attempt to eliminate the angular sensitivity seen with earlier versions of probe design. In order to determine if the reflector design was successful in eliminating the angular dependence of the ULBFP, the orientation of 2.5 mm dialysis tubing was varied from zero to maximum allowable misalignment with respect to the two transducers and the reflector bracket of the 8 mm perivascular ULBFP. Saline at 39°C was used both as a perfusate and the couplant material.

It may be shown (Appendix B) that since a constant wide planar acoustic wave is used, the vessel may be placed anywhere within the probes acoustic window and the device will measure the same volume rate of flow. To determine if the position of the vessel effects the flow reading, the position of the dialysis tubing in the acoustic window of the 8 mm perivascular ULBFP was varied and the output signal recorded. The vessel was placed horizontally through the probe with minimal misalignment. Five positions were chosen; centre, left and right of centre and above and below centre. Saline at 39°C was used as both the perfusate and the couplant material.

It may also be shown that the flow signal produced by the probe should be independent of the velocity profile of the flowing medium. To verify this, two sections of dialysis tubing were placed in the 8 mm perivascular ULBFP and the output signal was recorded with parallel and counter-current flow in the tubes. These two velocity profiles are much different from each other since one is symmetric and the other is asymmetric. They are also radically different than either the constant or pulsatile types of flow through a single section of tubing. Saline at 39°C was used as both the couplant media and the perfusate. For this experiment, pulsatile flow was used. The two vessels were centred in the sensing element of the probe.

3.5.5 Acoustic Couplant Material

Although no direct contact between the probes transducers and the vessel is required, a couplant material must be used to provide continuity between transducers. Several types of acoustic coupling materials were tested using the 4 mm perivascular probe in vitro (dialysis tubing) and in vivo (sections of intact femoral arteries). The couplant materials tested were isotonic saline, water, Nalco 1181⁸¹, a superabsorbant polymer, petroleum jelly, coagulated blood cells and air. The vessel or section of dialysis tubing was filled with blood or saline respectively and the signal strength measured by the T101 blood flow meter was recorded. The liquid couplants, saline and water, were employed by filling the cavity surrounding the vessel with the fluid such that the probe was totally submerged. The other types of couplant, with the exception of air, are gels and tend to flow away

from their application sites. To eradicate this problem a small latex purse (2 cm x 2 cm) was constructed to contain the couplant around the probe/vessel assembly⁸².

3.5.6 Signal Drift and Resolution

In order to determine the signal drift of each type of instrument, they were setup to measure a constant flow of 39.0°C saline over an eight hour period. The signal response to zero flow was monitored periodically over the entire experiment. The saline temperature was held constant by use of the heater stirrer bath and the heat exchanger. Medium temperature was monitored with type K thermocouples placed in the bath and in close proximity to the probe.

The resolution of the various flow measurement devices was determined by altering the flow from the rotary pump to find the smallest observable change in output voltage from each device.

3.5.7 Porous Media

A flow cell, shown in Fig. 21, was constructed to determine the thermal probe's ability to measure flow in porous media. The flow cell consisted of a perspex cylinder 25 cm long and had an inner and outer diameter of 1.5 and 1.9 cm respectively. Two holes on opposite sides of and midway down the cylinder, allow the thermal probe to be placed within the porous medium. Three media were used to fill the flow cell; coarse sand, fine sand and sponge. The temperature of the medium, isotonic saline, was varied from 34.0 to 45.0°C, for flow rates up to 120 ml/min. Flow rates above this were unattainable because of the resistance in the flow cell. This is the total flow rate through the flow cell and not the local flow rate in the vicinity of the probe. The explicit velocity profile across the cell is not known but is assumed to be zero near the side walls and maximal in the centre (approximately 25 ml/min/cm² assuming a parabolic profile).

The probes were placed in the flow cell and the zero flow reading was recorded to determine the voltage decay due to convection alone. For this test, both water and saline

Fig. 21 A cross-section of the flow cell used in conjunction with the TPD probes.

were used. In addition, both sand and sponge wetted with saline were examined. Tissue data from kidney, muscle, fat and spleen were taken at the time of death in one of the animal experiments. It was assumed that there would exist a flow gradient across the cell and that the probe may be able to distinguish this flow gradient. The decay constant of thermal probe #1 was measured for different flow rates of saline through the flow cell containing coarse sand. The position of the thermistor within the flow cell was varied from its central position (0 cm) to the inner edge of the cell wall (1.5 cm). The experiment was repeated for the other two porous media, fine sand and sponge.

Probe #1 was used to determine the effect of medium temperature on the decay constant of the probe when used in conjunction with porous media. Three saline temperatures were examined, 35, 39 and 45.0°C for a range of flow rate from 0 to 120 ml/min. The position of the probe was in the centre (0 cm) of the flow cell.

3.5.8 Calibration of TPD Probe in an Isolated Organ

If possible, a probe should be calibrated under conditions which closely resemble the in environment in which they are to be employed. In this case, an isolated organ and the tracer microsphere technique provide an ideal opportunity to calibrate the TPD probes. To this end, three female Yorkshire pigs, weighing between 30 and 40 kg, were prepared as indicated in Appendix D. The left kidney was isolated and 5000 UPS Heparin was injected intravenously prior to nephrectomy. The externalized kidney was perfused with a 4.0°C degassed isotonic saline solution containing 4% mannitol for 15 minutes. After flushing, the kidney was attached to the isolated organ apparatus, shown in Fig. 22 and perfused with 39.0°C saline. The temperature of the saline, monitored continuously with type K thermocouples, was held at the desired temperature using a combination of a heat exchanger and a constant temperature reservoir. Tygon tubing was used to connect the kidney and the reservoir to reduce air in the lines. The pulsatile pump was used to pump the saline perfusate through the organ.

Fig. 22 The isolated organ apparatus used to validate the thermal pulse decay probes and radioactive tracer microsphere techniques.

Three thermal pulse decay probes were placed into the inferior ventral surface of the kidney. The probes were introduced into the tissue with minimum trauma by making a small surface incision with a needle tip and slipping the probe through the entrance window created. There was little and in some cases no leaking at the surface using this method. The probes were placed 1.0 cm apart and penetrated 1.0 cm into the tissue.

The radioactive tracer microsphere technique was used to determine the local values of tissue perfusion and hence calibrate the TPD probe. For each of the three kidneys, six microspheres were used (^{57}Co , ^{113}Sn , ^{95}Nb , ^{85}Sr , ^{46}Sc , and ^{103}Ru) for flow rates ranging from 0 to 150 ml/min. Flow rates higher than this could not be tested since the kidney pressure was restricted to below 150 mmHg. The after vortexing for 5 minutes, the microspheres were injected on the arterial side of the kidney. An in line stirrer bar mixed the microspheres with the saline. The microspheres distribute themselves through the tissue in proportion to the flow and with the use of a reference syringe or "organ" the perfusion to a section of the organ or the entire organ is determined. It is the intention that the isolated organ calibration of the probes would be used to ascertain the perfusion values of the thermal probes when used in situ.

3.5.9 In Vitro Validation using an Isolated Organ

To calibrate the microsphere technique in vitro against known flow rates, an isolated organ and pulsatile pump are used. Three female Yorkshire pigs weighing between 30-40 kg, were prepared as per Appendix D. The left kidney was isolated and 500 UPS/kg Heparin was administered intravenously prior to the nephrectomy. The kidney was catheterized and a degassed 4°C saline/4% mannitol drip was used to flush the organ. This technique has been shown to maintain vascular structure of the kidney⁸³.

After 15 minutes, the organ was perfused with degassed isotonic saline at 39.0°C using a pulsatile pump. The outflow of the kidney was recirculated into the reservoir. A bubble trap was placed in line before the pump to removed any air bubbles. The microsphere injection (1 ml/s) site was between the pump and the arterial side of the

kidney. Since all of the microspheres would be directed to the single kidney, the standard microsphere solution was diluted by a factor of 10, such that approximately the same number of microspheres would be trapped in the kidney as would be trapped in situ. An in-line stirrer bar mixed the microspheres with the saline perfusate. Between the stirrer bar and the arterial side of the kidney, a reference organ syringe withdrew at a rate of 24.7 ml/min for 1 min starting 5 s before the injection time. The experimental configuration used for these isolated organ studies is shown in Fig. 22.

Kidney pressure was monitored throughout the procedure, and was maintained below 120 mmHg⁸³. The pressure was monitored with an in-line pressure transducer positioned on the arterial side of the organ. The flow rate, controlled by the pulsatile pump, was set and microspheres were injected at 15 minute intervals over a period of 1 to 2 hours for different flow rates. At the end of the experiment, the kidney was sectioned. The method used to determine the activity of the tissue and reference samples is outlined above.

The in situ experiments were used to determine the degree of microsphere mixing with the blood. The distribution of spheres in the isolated kidney should be similar to that in the in situ kidney provided that the flow distribution was homogenous in both and that the vasculature of the isolated kidney was grossly comparable to the in situ kidney. To verify this, each was sliced from superior to inferior into 7-8 1.5 cm wide sections. For each section, the activity and perfusion were determined.

3.5.10 In Situ Measurements

One assumption of the RTM technique is that the microspheres are well mixed. In addition, microspheres should not result in abnormal changes in the circulation and should be trapped in the tissue after a single pass through the microvasculature.

To assess the validity of these assumptions, swine were prepared surgically in the manner outlined for the isolated organ experiments (Appendix D) with the exception that the left kidney was not removed. The left ventricle, left femoral, right atrium, and bladder were

all cannulated. The left kidney was exposed and a perivascular ultrasonic flow probe was placed over the renal artery. The animal was left to stabilize for a period of one hour. Microsphere samples (10ml) were injected (1ml/s) into the left ventricle and flushed with isotonic saline (10ml) six times over the next two hours. The reference organ sampled arterial blood from the left femoral artery at a rate of 24.7 ml/min for a period of 1 minute. The electrocardiogram, left ventricle pressure, central venous pressure and essential blood gases (pH, pCO₂, and pO₂) were monitored throughout the experimental protocol.

At the end of the experiment, the animal was sacrificed by injection of concentrated potassium chloride (KCl) solution into the left ventricle. The arterial and venous blood samples, as well as the left and right kidney samples were counted. The counting procedure is outlined previously in section 3.4.

If upon injection, or shortly thereafter, the microsphere solution caused a significant change in central venous pressure, the ECG or the left ventricle pressure, the data from that point were discarded.

The degree of homogeneity (mixing) of the microsphere distribution in blood can be assessed by comparing the flow through pairs of organs such as the hemispheres of the brain or the left and right kidneys^{57,84}. Proper mixing should result in equivalent flow to the left and right kidneys. Before any major manipulation of the kidney, the first microsphere suspension is injected. After the experiment is over, the animal is sacrificed, the left and right kidneys removed, counted and then compared to assess their initial flows. A total of 11 kidney pairs were used in this analysis.

To determine if the microspheres were trapped on the first circulation through the organ, an autoperfused kidney was used. The left kidney is externalized, placed in a water bath and supplied with whole blood from the left femoral artery. The venous outflow of the kidney was returned to the animal through the left femoral vein. Microspheres were not

injected until the kidney had begun to produce urine, an indicator of renal function. Upon injection of microspheres both the arterial and venous blood were sampled at a rate of 24.7 ml/min. This was repeated for all six of the microsphere suspensions over a 1 to 2 hour period. The animal was sacrificed and after dissection, the kidney and venous and arterial blood samples were counted to determine the flow and the trapping efficiency of the kidney.

4. Results

4.1 Electromagnetic Flow Probe Results

4.1.1 Type of Flow

Fig. 23 shows the EMBFM signal (voltage) as a function of flow rate for 39.0°C for two types of flow, pulsatile and constant through the 4 mm cannulating EMBFP. The two data sets are fit to the function $y=ax+b$ and are given as $y=(4.50\pm0.34)x+(6\pm10)$ and $y=(4.66\pm0.37)x-(25\pm50)$ for pulsatile and steady flow respectively. As can be seen, the steady flow calibration has a slightly steeper slope and a larger zero flow offset.

4.1.2 Temperature of the Medium

The response of the 4 mm cannulating EMBFP to a steady flow of saline at three temperatures is shown in Fig. 24. The data are fit to 1st order polynomials with the following result; $y=(4.59\pm.30)x-(20\pm20)$, $y=(4.67\pm0.31)x-(25\pm20)$ and $y=(4.67\pm0.27)x-(6\pm17)$ for 35.0, 39.0, and 50.0°C respectively.

The response of the same cannulating probe for pulsatile flow at three temperatures of saline for a range of flow rate from 0 to 250 ml/min is shown in Fig. 25. The resulting linear parameterization of the data for 35.0, 37.0 and 47.0°C are $y=(4.45\pm0.15)x+(11\pm12)$, $y=(4.50\pm0.21)x+(6\pm10)$, and $y=(4.52\pm0.25)x+(5\pm12)$ respectively.

The parameterization for the blood-saline mixture flowing through the 4 mm i.d. cannulating EMBFP at three temperatures is shown in Fig. 26, where the recorded output voltage is plotted against the rate of flow. The equations of the linear fits are $y=(5.19\pm0.33)x-(40\pm20)$, $y=(5.19\pm0.37)x-(37\pm25)$, and $y=(5.56\pm0.25)x-(44\pm45)$ for 35.0, 39.0 and 45.0°C respectively. The value for the zero offset for the blood substitute is consistently large and negative.

Fig. 23 Output voltage of the 4 mm cannulating electromagnetic flow probe is plotted against the flow of saline for steady and pulsatile flows at 39°C.

Fig. 24 Output voltage of 4 mm diameter cannulating electromagnetic flow probe plotted against steady flow of saline for several temperatures.

Fig. 25 Output voltage from 4 mm cannulating electromagnetic flow probe plotted against pulsatile flow of saline for several representative temperatures.

4.1.3 Type of Medium

In Fig. 26, the output voltage from the 4 mm cannulating EMBFP is plotted against pulsatile flow for isotonic saline at 39.0°C and the blood substitute at several temperatures. The linear fit equations for the two media at 39.0°C are $y=(5.19\pm0.20)x-(37\pm25)$ for blood and $y=(4.50\pm0.31)x+(6\pm10)$ for saline. Comparing the fit equations, blood has steeper slope and a larger zero offset. At high flows there is a 12% difference between the output voltage for saline and that of the blood-saline mixture at the same temperature.

The output voltage of the same cannulating probe is plotted against pulsatile flow of isotonic saline, the blood-saline mixture and fresh whole swine blood at 39°C. The data are shown in Fig. 27. Compared to saline, the linear fit to the data for the blood substitute is 12% higher and the whole blood is somewhat lower at 5%. The fits to the data for the blood substitute and saline are given above. The linear fit to the data representing whole blood is $y=(4.20\pm.33)x+(28\pm43)$. The slope for wholeblood is shallower than the slopes for either the blood substitute or isotonic saline.

4.1.4 Vessel Type

Since inorganic vessel act as insulators only freshly denervated swine aorta were good conductors and could be used in vitro to calibrate the 8mm perivascular electromagnetic probe. In Fig. 28, the output voltage of the probe is plotted against the pulsatile flow of blood and saline at 39.0°C. The fits to the data are, for saline, $y=(6.43\pm0.33)x-(2\pm10)$ and for blood, $y=(7.11\pm0.38)x+(13\pm13)$. As can be seen, the slope for blood is steeper and has a larger zero flow offset.

4.1.5 Signal Drift and Resolution

The time dependence of the signal received from the cannulating probe is shown in Fig. 29. This was determined by monitoring the change in the zero flow signal over a period of eight hours. Saline at 39.0°C was used as a perfusate. As can be seen, there

Fig. 26 Output voltage for 4 mm cannulating electromagnetic flow probe plotted against pulsatile flow of blood-saline at three temperatures. The signal for saline at 39.0°C is plotted against flow for comparison.

Fig. 27 The output voltage of the 4 mm cannulating probe is plotted against the pulsatile flow of saline, a blood-saline mixture and whole blood all at 39.0°C.

Fig. 28 Output voltage of 8 mm perivascular electromagnetic flow probe plotted against the pulsatile flow of blood and saline at 39.0°C.

Fig. 29 The signal drift for zero flow is shown over a period of eight hours. The 4 mm cannulating electromagnetic probe was used with degassed isotonic saline at 39.0°C.

is very little drift in the signal after approximately one hour. The large error bars shown in the figure are a result of the reading error of the stripchart recorder.

4.2 Transit Time Flow Probe Results

4.2.1 Type of Flow

The measurement (output signal) of the perivascular ULBFP due to the flow of isotonic saline through a 2.5 mm diameter section of dialysis tubing is shown in Fig. 30. For steady and pulsatile flows ranging from 0 to 250 ml/min, the output signal of the 4 mm probe is plotted for saline at 39°C. The data are fit to the linear function $y=ax+b$. For pulsatile and constant flow the resulting equations are: $y=(3.24\pm0.15)x-(15\pm10)$ and $y=(3.29\pm0.15)x-(7\pm6)$ respectively. As can be seen, the slope for pulsatile flow is slightly steeper and the zero offset. There is less than a 1% difference in the recorded output voltages between steady and pulsatile flow.

4.2.2 Temperature of Perfusate

In this experiment, saline at different temperatures was pumped through 2.5 mm dialysis tubing. The voltage response of the 4 mm perivascular probe placed around the vessel was not measurably effected by temperature changes over the range 35 to 45°C. The signal recorded when the blood/saline mixture was used as a perfusate did display a small temperature dependence. The change in signal for a 10°C change was 3%.

4.2.3 Type of Perfusate

In Fig. 31, the output voltage of the 8 mm perivascular ULBFP placed over 2.5 mm dialysis tubing is plotted against the flow rate of saline and a blood-saline mixture at 39.0°C. The couplant material was isotonic saline at 39.0°C. Using a linear fit to the data, the equation for blood is $y=(2.62\pm0.17)x-(24\pm15)$ and $y=(2.74\pm0.10)x-(4\pm5)$ for saline. At 250 ml/min there is an 8% difference in the output signal between the blood-saline mixture and isotonic saline. The fit for saline has a steeper slope, as well as a much smaller zero flow offset.

Fig. 30 Steady and pulsatile flow of isotonic saline at 39.0°C through a section of 2.5 mm i.d. dialysis tubing measured by the 4 mm perivascular ultrasonic flow probe.

Fig. 31 The voltage output of the 8 mm perivascular ultrasonic flow probe over 2.5 mm dialysis tubing is plotted against flow of saline and blood at 39.0°C. Isotonic saline is used as a couplant material.

Fig. 32 Calibration of the 4 mm perivascular and cannulating ultrasonic flow probes both in vitro and in vivo. Saline was used as the couplant.

The response of the 4 mm cannulating and perivascular probes to pulsatile flow is shown in Fig. 32. These four sets of data are calibration curves for these instruments both in vitro and in vivo. The in vivo data are for whole blood from the left femoral artery of the pig. The cannulating probe was calibrated in vitro with saline. The perivascular probe was calibrated in vitro around a section of swine aorta using saline as both the couplant and medium. The data may be parameterized with linear plots. For the cannulating probe in vitro, the fit to the data is $y=(2.92\pm0.13)x-(19\pm11)$ and $y=(3.08\pm0.15)x-(3\pm5)$ in vivo. The fits to the data from the perivascular probe are $y=(3.35\pm0.13)x-(16\pm10)$ and $y=(3.75\pm0.13)x+(1\pm6)$ for in vitro and in vivo respectively. For both types of probe the in vivo fit to the data has a steeper slope and smaller zero flow offset than the in vitro fit

4.2.4 Couplant Material

Several acoustic couplant materials were used with the perivascular ULBFPs both in vivo (sections of femoral artery) and in vitro (2.5 mm dialysis tubing). The couplants tested were isotonic saline, Nalco 1181, petroleum jelly, air and coagulated blood. Saline had the highest signal strength both in vivo and in vitro. The couplant with the lowest signal strength, (other than air), was coagulated blood. The data are shown in Fig. 33.

4.2.5 Vessel Type, Orientation and Position

Several vessels were placed in the acoustic window of the perivascular ULBFP. In all cases isotonic saline was used as both the couplant and the perfusate. The signal strength from the probe was recorded for each of the vessel types. The results are shown in Fig. 34. The vessels tested were, Masterflex 6411-17,16,15,14, Tygon, Latex (4 mm o.d.), 2.5, 6.0 and 6.8 mm Spectrum dialysis tubing, and sections of unfixed swine aorta. Dialysis tubing and aorta had the highest signal strength, with dialysis tubing only slightly better. The other vessel types displayed a somewhat reduced ability to transmit ultrasound.

Fig. 33 The signal strength as measured by the T101 blood flowmeter relative to the signal for saline is shown for each of the couplant materials in vitro over dialysis tubing and in vivo over the left femoral artery of the swine.

Fig. 34 The signal strength recorded by the T101 blood flow meter relative to dialysis tubing is plotted for a number of vessel types.

A section of 2.5 mm dialysis tubing was placed in the 8 mm perivascular probe. The signal response of the probe was measured for a range of flow from 0 to 250 ml/min. The tubing was then replaced with 6.8 mm dialysis tubing and the experiment repeated. The data, as shown in Fig. 35, may be fit with linear functions. The resulting equations are $y=(2.61\pm0.20)x-(23\pm15)$ and $y=(2.74\pm0.23)x-(5\pm6)$ for 2.5 mm and 6.8 mm dialysis tubing respectively. The 6.8 mm dialysis tubing gave a 7% larger signal. In addition, its fit has a steeper slope and smaller zero flow offset. When the dialysis tubing was replaced by a section of fresh, unfixed swine aorta, the signal response of the perivascular probe was again linear with the flow rate. The data may be fit to the linear equation, $y=(2.48\pm0.15)x-(3\pm6)$. The signal for aorta and 2.5 mm dialysis tubing differ by as much as 2%. A 6.0 mm diameter section of dialysis tubing (not shown) gave a similar result. For the 6.5 mm diameter dialysis tubing, the output voltage of the probe was larger than that for the smaller diameter tubes.

In order to study the effect of vessel position with respect to the transducers and reflector, a 2.5 mm dialysis tube was placed in several positions. The five positions tested are illustrated in the insert of Fig. 36. When fit with linear functions, the central positions 2, 5, and 3 could be fit by the following, $y=(2.53\pm0.13)x-(15\pm11)$, $y=(2.61\pm0.15)x-(23\pm12)$ and $y=(2.39\pm0.13)x-(11\pm7)$ respectively. Position 5 had the steepest slope and the largest zero flow offset, whereas position 3 had the smallest slope and smallest offset. Positions 2 and 5 differ by less than 2% in their output responses, and have similar slopes and zero offsets. Position 3, closest to the reflector bracket, had a 5% lower response than the centre position (2). Positions 1 and 4, to the left and right of centre, showed a much lower response. The fit equations for these two positions are for position 1, $y=(1.75\pm0.10)x-(15\pm8)$ and $y=(1.86\pm0.11)x-(7\pm9)$ for position 4. These off-centre positions have slopes which are much lower than the central ones.

Using 2.5 mm dialysis tubing in the 4 mm perivascular probe, the effect of vessel orientation in the sensing volume of the probe was tested. Saline at 39°C was used as both the couplant material and the perfusate. In Fig. 37 the effect of vessel alignment on

Fig. 35 The output voltage of the 8 mm perivascular flow probe is plotted against pulsatile flow for several diameters of dialysis tubing and a section of swine aorta. Saline at 39°C is used as a perfusate.

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Fig. 36 The output voltage of the 8 mm perivascular probe is plotted against pulsatile flow of 39°C saline for several positions in the sensing volume of the probe. The positions are shown in the insert.

Fig. 37 Vessel (dialysis tubing) orientation with respect to the transducers and reflector bracket was varied from minimum to maximum misalignment as shown in the insert. Saline at 39°C is the perfusate and couplant material.

flow measurement is shown and the insert of the same figure shows the positions of minimum and maximum misalignment in the probe. The data in the figure may be fit with linear functions. The fits to the data are, $y=(3.35\pm0.11)x-(14\pm12)$, for the minimum and $y=(2.92\pm0.10)x-(25\pm15)$ and $y=(2.92\pm0.09)x-(23\pm17)$ for the 1 and 2 maximum misalignment positions respectively. The positions of maximum misalignment show a decrease in the measured output signal compared to the zero misalignment position.

Using saline at 39°C for both the couplant and perfusate, two sections of 2.5 mm dialysis tubing were placed in the 8 mm perivascular probe. The response of the probe to a range of flows from 0 to 125 ml/min was measured for parallel and counter-current flow in the vessels. The calibration of the probe using a single section dialysis tubing and saline was used to analyze these data. For counter-current equal flows, the ultrasonic flow meter recorded zero flow. For parallel equal flows the probe measured twice the flow through a single section of dialysis tubing.

4.2.6 Signal Drift and Resolution

The 4 mm perivascular probe was placed around a section of dialysis tubing. Saline at 39.0°C was used as both an acoustic couplant and a perfusate. Over an eight hour period the drift of the zero flow reading of the 4 mm perivascular probe, shown in Fig. 38, was 20 mV. Using the calibration of this probe with dialysis tubing and saline, 20 mV corresponds to a 6 ± 2 ml/min drift. The smallest flow which could be resolved was 5 mV, or 2 ml/min. After a period of approximately one hour there was very little change in the output signal.

4.3 TPD Probe Results

4.3.1 Type of Flow

Using distilled degassed water at 39.0°C, the decay constant of the thermal pulse decay probe in a T type connector was determined for both steady and pulsatile types of flow. Flow was varied from 0 to 120 ml/min. The data for steady and pulsatile flow are shown in Fig. 39 for probe #1 and parameterized with fourth order polynomials. There is less

Fig. 38 The drift of the ultrasound signal is shown over an eight hour period. The 4 mm perivascular probe was used in conjunction with 2.5 mm dialysis tubing and saline.

Fig. 39 The decay constant of probe #1 for different flow rates is shown for pulsatile and steady flow. The probe is in a T-type junction and the medium is water.

Fig. 40 The decay constant of the thermal probes is shown versus the rate of flow of water through the T-type connector.

than 1% difference between the calibration curves for pulsatile and steady types of flow for flow rates less than 120 ml/min. The accuracy of this TPD probe (#1) is approximately 15% for high flow rates. Note that this is for the probe positioned in the cannulating T connector only. The resolution of the probe is given as 10 ml/min.

4.3.2 Type of Perfusate and Perfusate Temperature

Two different perfusates were tested in conjunction with the thermal pulse decay probes; degassed isotonic saline and distilled water. The decay constant of the four thermal probes is shown in Fig. 40 for water at 39.0°C and flows from 0 to 120 ml/min. The data for the four probes may be parameterized with fourth order polynomials. Probes #1 and #2 have significantly better fits to the data than the other two probes. The decay constants for probes #3 and #4 do not decrease as much as the decay constants for the other two probes over the same flow rate.

Utilizing probe #1, the comparison between the calibration curves for water and saline at the same temperature may be seen in Fig. 41. The decay constant for zero flow for saline (-2.21 ± 0.03) is much lower than that for distilled water at the same temperature (-1.96 ± 0.02). The curve for saline has consistently lower decay constants for a given flow than the curve for water.

The constant temperature reservoir, used in conjunction with the in-line heat exchanger, was used to alter the medium temperature. The decay constant of the thermal probe was determined for the steady flows from 0 to 180 ml/min for three temperatures: 35, 39, 45°C. The zero flow decay constants for saline were again consistently lower (-2.23 , -2.21 , -2.20) than the zero flow decay constant for water (-1.99 , -1.96 -1.93). There is an increase in the zero flow decay constant as the thermal background temperature of either the water or saline increases.

Fig. 41 The time constant of Probe #2 versus flow is shown for 39.0°C water and saline.

Fig. 42 Three temperatures of water are used to calibrate probe #1 against temperature. The decay constant for different steady flows is shown.

Fig. 43 The decay constant of Probe #1 for different flow rates using saline is shown for three temperatures.

4.3.3 Porous Media

Using saline as a perfusate, the probes were inserted into the flow cell packed with either fine or coarse sand or sponge material. To determine the effects of convection apart from diffusion, the voltage decay of the probe was measured in the porous media with no perfusion. The data, all at 39.0°C, for fine and coarse sand, sponge, water and saline are illustrated in Fig. 44. The decay constants for various tissue samples are shown in the same figure. The samples tested were kidney, muscle, fat and spleen. Sponge had the highest decay constant of the inorganic materials. Both types of sand (fine and coarse) and saline had the lowest zero flow decay constants (-2.20 ± 0.02) kidney, water and spleen had approximately the same values (-1.96 ± 0.03). Sponge and muscle were also similar. Fat was the highest zero flow decay constant at (-1.09 ± 0.02). The term 'zero flow' decay constant refers to the fact that the pump is off. Effects, such as thermal gradients may cause small local flow variations in the vicinity of the probe, but it is assumed that these effects are small and do not significantly alter the heat dissipation about the TPD bead.

The position of thermistor probe #1 was varied across the diameter of the flow cell. For the same flow rate, the measured decay constant as a function of the position from the centre of the cell is shown in Fig. 45 for fine and coarse sand, as well as, the sponge material. The data are fit with curves merely to guide the eye and no physical significance is implied. The decay constant of the measured data increases as the position of the TPD probe is varied from its central position to the end of the flow cell indicating a flow gradient across the cell.

The decay constant of probe #1 was determined for different flow rates when the thermal probe was placed in the flow cell containing coarse sand. The probe is positioned in the centre of the cell at 0 cm. Three saline temperatures: 35, 39 and 45.0°C were investigated for a range of total flows through the cell from 0 to 120 ml/min. The data may be parameterized with polynomials.

Fig. 44 The decay constant of probe #1 for different media are shown for zero flow rate.

Fig. 45 The decay constant of probe #1 is shown as a function of position in the flow cell for three media. The flow rate (approximately 50 ml/min through the cell) is the same for all media tested.

Fig. 46 The decay constant of probe #1 is plotted against the flow of saline through the flow cell at different temperatures. The porous medium used is coarse sand and the probe is positioned in the centre of the cell.

4.3.4 Isolated Organ Perfusion Studies

When the thermal pulse decay probes were used in the isolated saline perfused swine kidneys, the decay constants of the data were not significantly different from the zero flow values found in the in vitro experiments. In Fig. 47 the decay constant determined from curve fitting is plotted against the value of perfusion determined from the radioactive tracer microsphere technique. Since the data set is for low perfusion values, a linear parameterization of the data may be used. Although the decay constants over a large range of flow rates are nonlinear, the flow over a very small region may be approximated by a linear fit. The linear fit, $y = -(0.23 \pm 0.09)x - (1.9 \pm 0.2)$ does not approximate the data very well, but the trend in the data is clear. The measured decay constant decreases as the local value of perfusion in the probe increases. This is consistent with the trends seen in the in vitro experiments with the cannulating TPD probe and the data from the flow cell.

4.4 RTM Results

4.4.1 In Vitro Experiments using an Isolated Organ

Flow through the kidneys was controlled with a rotary pump. For each of the kidneys, six different radiolabeled microspheres were injected. The flow rates ranged from 0 to 150 ml/min. Higher flow could not be achieved since the pressure had to be maintained below 120 mmHg. After all the microspheres have been injected the sample was dissected and counted in the Ge(Li) detector to determine its activity. A typical spectrum for a section of a left kidney is shown in Fig. 48. Note that the six isotopes used in the experiment are readily identified by their large peaks. This spectrum is passed to the NRC program D40 which determines the peak areas and mean peak energies. By comparing the peaks of the sample and reference spectrums it is possible to determine the perfusion to the sample using equation [41]. Tissue data from three saline perfused swine kidneys are shown in Fig. 49. The perfusion of these kidneys (determined from the activities in the spectra) is plotted against the known flow of the pulsatile pump. The data may be parameterized with the linear equation, $y = (1.16 \pm 0.26)x - (11 \pm 15)$.

Fig. 47 The decay constants of thermal probe #1 are plotted against the values of perfusion determined by the microsphere method. The data from three saline perfused kidneys are shown.

Fig. 48 The gamma spectrum of a section of swine kidney (Weight=10 g). The peaks are identified by their energies. The area under the peak is the activity of the sample.

Fig. 49 Using isolated saline perfused kidneys, the total flow through the kidney was calculated by the RTM technique and is calibrated by the rotary pump.

Referring to Fig. 50, the perfusion profile measured across the isolated kidney samples from superior to inferior sides indicated that the microspheres distributed themselves in the same manner as the microspheres within the in situ kidneys.

The kidney pressure was monitored throughout the experimental protocol with a pressure transducer and hence it is possible to determine the effect of successive microsphere injections on vascular resistance. There was no measurable increase in kidney pressure after a microsphere injection in all three of the isolated kidneys. A strip recording in Fig. 51 is typical of these experiments, with the exception that the ECG trace is absent.

4.4.2 In Vivo Validation

To verify some of the assumptions of the radioactive tracer microsphere method, several experiments were preformed. The arterial and venous blood flowing through an autoperfused kidney were sampled (24.7 ml/min) for the duration of several microsphere injections. The detector system employed did not detect any radioactivity due to the microspheres in the venous blood.

By monitoring the vital signs during an injection, the physiological effects of a microsphere injection may be determined. If during, or shortly after, an injection there was a marked change in the ECG, central venous or left ventricle pressures then the data from the injection were discarded. The occurrence of such an effect was only seen on the first injection. A typical stripchart recording of an injection is shown in Fig. 51. Note that immediately after the injection and flush the left ventricle pressure returns to its preinjection position. Blood flow (measured by transit time ultrasound), central venous pressure and the electrocardiograph are not affected by the injection of the microspheres.

When a microsphere injection did affect the circulatory system, there was a marked drop in left ventricle pressure accompanied by a significant increase in the heart rate of the animal.

Fig. 50 Perfusion profile calculated by the RTM technique from in vitro and in vivo kidneys shown from superior to inferior.

Fig. 51 The ECG, transit time ultrasound flowmeter (UL), left ventricle (LVP) and central venous (CVP) pressures are monitored during a microsphere injection.

Since pairs of organ, such as the kidneys, should have similar flow rates, there should be equal concentrations of microspheres in both the left and right kidney. The data from the first injection of 11 experiments are shown in Fig. 52. Left and right kidney flow are equal to within 10% over the range of flows observed. The linear parameterization of the data can be written as $y=(1.06\pm0.22)x-(13\pm33)$. This indicates a high degree of mixing.

Fig. 52 Initial flow to the left and right kidneys of several pigs determined by the RTM technique.

5. Discussion

5.1 Electromagnetic Flow Probes

The difference in the EMBFP flow signal between steady and pulsatile flow of the medium was determined to be negligible. The flow signal was also determined to be linearly proportional to the volume rate of flow. For a given volume rate of flow, the signal was the same for both the steady and pulsatile experiments which is in agreement with Corver's⁸⁵ *in vitro* experiments. The pulsations produced by the pulsatile pump are integrated by the device, such that the instrument is measuring the average flow rate. Corver stated that the EMBFP were one of only a few flowmeters which actually record average flow, as opposed to fluid velocity. The independence of the signal on velocity profile holds only if the fluid's profile is axially symmetric. When the velocity profile is asymmetric, such as in streaming flows, the voltage generated can differ by a factor of two. If the flow is concentrated near the side walls, away from the electrodes, then it would lower, and higher if concentrated near the electrodes^{86,87,88}. Bevir¹⁷ briefly discussed non-axially symmetric flows. He determined that EMBFPs which would not be affected by asymmetries in the velocity profile were impossible to make with point electrode flow meters. A three dimensional varying magnetic field would be required, but would be impractical if not impossible to construct. In practice, the velocity profile *in vivo* should be symmetric provided that the measurement site is far enough away from any major bifurcation or the heart. By comparing the output voltage for constant flow and pulsatile flow *in vitro*, shown in Fig. 23, the difference is less than 2%, significantly less than two. This suggests that the flow provided by the pulsatile pump did not have an asymmetrical velocity profile, although this was not measured. Since the difference between steady and pulsatile flow profiles is small there will be no correction made to the output signal for flows *in vivo*. It will be assumed that all *in vivo* flows are axially symmetric and that the knowledge of the velocity profile is unimportant.

The difference between the measurement of flow for blood and saline is interesting and may be attributed to the different conductivities of these two fluids. From section 2.1, the ratio of the two voltages (saline/blood) for a given flow may be determined. The magnetic field and flow rate terms cancel leaving the term N involving the conductivity. If the wall conductivities are assumed to be the same, the ratio of N_{saline} (N_s) to N_{blood} (N_b) may be expressed as,

$$\frac{N_s}{N_b} = \frac{\sigma_s}{\sigma_b} \left[\frac{\frac{\sigma_b}{\sigma_w} \left(1 + \frac{a^2}{A^2} \right) + \left(1 - \frac{a^2}{A^2} \right)}{\frac{\sigma_s}{\sigma_w} \left(1 + \frac{a^2}{A^2} \right) + \left(1 - \frac{a^2}{A^2} \right)} \right] \quad [36]$$

From Gessner¹⁴, the aorta wall is approximately four times less conductive than blood, i.e. $4\sigma_w = \sigma_b$. Assuming that the device is around a section of aorta, stripped of fat and connective tissue and using Gessner's approximation, then with this substitution the ratio becomes,

$$\frac{N_s}{N_b} = \frac{4 \left(1 + \frac{a^2}{A^2} \right) + \frac{\sigma_s}{\sigma_b} \left(1 - \frac{a^2}{A^2} \right)}{5 + 3 \frac{a^2}{A^2}} \quad [37]$$

From Woodcock¹⁸, $\sigma_b = 0.6\sigma_s$ at 40% haematocrit and 37°C. ($\sigma_b = 6 \text{ mohm}^{-1}\text{cm}^{-1}$ and $\sigma_s = 9.6 \text{ mohm}^{-1}\text{cm}^{-1}$)⁸⁹ and the ratio a/A is typically .85. Under these conditions the ratio N_s to N_b is approximately 0.96. The difference between the voltage recorded for saline and that for blood for a given volume rate of flow is illustrated in Fig. 27 and Fig. 28 for the cannulating and perivascular probes respectively. The difference in the measured flow between blood and saline for the cannulating probe is 12%, while for the perivascular probe the difference is 10%. For the perivascular probe only the discrepancy between the expected and measured ratios of the signals, 14%, may be attributed to the

blood saline solution. This solution, commonly used in isolated heart studies, is reported to have the electrical properties of whole blood. The electrical conductivity of the medium used may have been significantly altered due to aging, however no data are available.

The difference in signal for the cannulating probe (16%) cannot be explained in terms of the conductivity of the medium alone. When the cannulating probe is used there is no aorta, hence the inner and outer wall thickness are equal and the ratio N_s/N_b , expressed in equation [37], is one. There can be no difference due to medium conductivity with zero wall thickness. The increased signal for the blood substitute relative to saline may be due to the formation of a thin film or boundary layer. The ratio N_s/N_b , determined by Gessner¹⁴, for a boundary layer of thickness ΔA and conductivity σ_{layer} in the absence of a wall, can be written as

$$\frac{N_s}{N_b} = \frac{1 + \frac{\Delta A}{A} \frac{\sigma_{\text{layer}}}{\sigma_b}}{1 + \frac{\Delta A}{A} \frac{\sigma_{\text{layer}}}{\sigma_s}} \quad [38]$$

He assumed that the layer would be between 1 and 5% of the vessel radius and that the layer was 1.5 times more conductive than whole blood and using his figures, the ratio of the signals varies between 1 and 3%. This accounts for some the discrepancy observed between the measured and calculated difference for the signals for blood and saline. In the course of the experiments the cannulating probe required continual cleaning to remove blood clots and tarnished electrodes. It is therefore a good assumption that there was a fluid layer surrounding the electrodes. The large difference in signal still has not been totally accounted for, but it is likely due to the electrical properties of the blood substitute.

To properly calibrate the probe, saline or fresh whole blood should be used. Using whole blood, the difference between blood and saline is much less. The literature accompanying the electromagnetic flow meter stated that in vitro calibration with saline would result in true flow reading when used in vivo. This is not the case since a 4% error in the output voltage would result. The difference in the conductivities of the two media cannot therefore be ignored. For in vivo use, the calibration with whole blood will be used, whereas in vitro the calibration for saline at 39°C will be used.

A linear parameterization of the data for whole blood was used even though a 2nd order polynomial would have given a better approximation to the data, since there is no theoretical basis for a higher order fit.

Changing the temperature of the medium alters its conductivity. The dependence of the output signal on the temperature of the medium was shown to be experimentally small. Referring to Fig. 24, where the output voltage of the 4 mm cannulating probe is plotted for steady flow of saline at three temperatures, the deviation of the signal from 20.0 to 50.0°C is 5% and the difference in signal between 35 and 50°C is 2%. For pulsatile flow, as seen in Fig. 25, the change in the output signal due to a change in temperature is 2% over the range 35.0 to 44.0°C. The change in signal, shown in Fig. 53, due to a change in media temperature is small. This is expected since the conductivity of the medium over this range is not strongly affected by temperature¹⁸. Since in vivo the temperature of the blood, (measured by a thermocouple), did not change by more than $\pm 2^\circ\text{C}$, and the physiological temperature of blood is unlikely to be outside of very small limits ($\pm 4^\circ\text{C}$), no correction for temperature was required.

In clinical use, it is still necessary to monitor the basal temperature in order to determine if the temperature exceeds an acceptable limit and thus requires a modification of the flow signal produced by the electromagnetic probe. Examples of this are a hyperthermia treatment in which blood temperatures may reach 47-50°C and during certain surgical

Fig. 53 Using the cannulating probe, the output signal for a given volume rate of flow of saline is shown as a function of temperature.

procedures where hypothermia is induced, reducing the core body temperature to about 10°C.

The necessity for an accurately fitting flow probe is evident since no output signal is recorded for smaller diameter vessels. Precise perpendicular placement of the perivascular probe is essential for good electrode contact. These two conditions make the use of a given flow probe limited and necessitate that the researcher have several flow probes of varying diameters. In addition, each of these probes would require the same calibration procedures as described above.

The rigid cuff which houses the two electrodes and the magnet in the perivascular probes, has a cylindrical centre so that it may be placed around a vessel. In acute applications the probe must actually constrict the vessel in order to maintain electrical contact. The effect of constriction in acute applications can alter right ventricular pressure if the measurements are made in close proximity to the heart⁹⁰. Constriction may also dramatically alter the flow profile of the perfusate across the vessel. This may be seen by examining Bernoulli's equation⁷⁸ which may be written as,

$$\frac{1}{2}\rho v^2 + p = K_B \quad [39]$$

where p pressure and K_B is Bernoulli's constant. A decrease in pressure will cause an increase in the velocity. This increase in velocity is not a problem in terms of measuring flow since these probes measure volume flow and not velocity profile. In chronic applications, constriction is not necessary since the surrounding tissue fibroses and establishes sufficient contact with the electrodes. If the probe is placed near the heart, it is more susceptible to electrical noise from the ECG and eddy currents in the flow⁹¹. In the in vivo experiments performed in this investigation, the probe was placed on the renal artery and is considered far from the heart and suitable for the assessment of

regional blood flow in the peripheral circulation⁹². Therefore, no corrections to the recorded output signal due to probe location are required.

The signal drift of the EMBFP over a period of eight hours is shown in Fig. 29. After a warm up period of one hour the average signal does not change. There is very little drift in the signal and the error in the signal is due to the resolution of the stripchart recorder paper and the inherent resolution of the pump. The smallest resolvable flow was limited to the resolution of the rotameter and chart recorder which combined are 5 ml/min.

5.2 Transit Time Flow Probes

It was shown in section 2.2 that the transit time ULBFP signal is independent of the velocity profile of the medium. The signal for the steady and pulsatile flow experiments, shown in Fig. 30, were almost identical. It was assumed that the velocity profiles of the two types of flow were axially symmetric, but had different radial profiles. The pulsatile pump was assumed to have produced a turbulent profile, whereas the steady flow was assumed to be laminar. A future experiment should include using laser doppler techniques to measure these profiles. If the assumptions are correct then the transit time probes are capable of measuring flow independent of the velocity profile of the medium. The velocity independence of the transit time probe has been well documented in the literature and demonstrated by a number of authors^{93,94,95,96}.

One of the experiments dealing with position of vessels within the probe window validates the velocity independent aspect of the probes. Two vessels of 2.5 mm dialysis tubing were placed together within the 8 mm perivascular probe. This configuration is by construction not radially symmetric. In addition, the ULBFP correctly measured the rate of flow for both counter-current and parallel flows. This clearly supports the postulate that the ULBFP can measure the total outflow independent of velocity profile. This also implies that the probe may be used over a bifurcation. However, when used over an artery-vein pair the reported flow would be the net difference between the artery

and vein. Since there is little difference in the output signals for any of the flow types measured with the transit time ultrasound probes, no correction to the output signal for velocity profile will be made.

The signal strength of the acoustic beam was measured with saline as a couplant. Several types of tubing were placed in the probe and the signal strength was noted. Several of the tubes caused the ultrasound beam to be scattered or attenuated such that the signal strength was too low to be monitored. Dialysis tubing allowed the propagation of the signal and resulted in the highest signal strength. It was therefore used in most in vitro investigations. Sections of aorta were removed from swine at death and the attached to the perfusion apparatus. The signal strengths of the flow meter for the dialysis tubing and freshly denervated arteries were within 10% of each other. This suggests that dialysis tubing could be a suitable vessel-equivalent material for studies in vitro which require repeated measurements to be taken. The advantage would be that the dialysis tubing would not decay over a period of a few days and no animal sacrifice would be required.

Dialysis tubing of varying diameter (2.0, 6.0, 6.5) were placed in the probe. For a given volume rate of flow, the output signal was found to be independent of tube diameter. This is consistent with the theory of section 2.2 and implies that the vessel diameter need not be measured. This observation is also consistent with the position that the signal is independent of the velocity profile, since the velocity profile must change with tubing diameter for a given flow.

Using a section of 6.8 mm diameter dialysis tubing, a larger output voltage was recorded than for the smaller diameter tubing for the same flow. This is likely due to the construction of this particular section of tubing. A large diameter of dialysis tubing was wrapped around itself such that the inner diameter was now 6.8 mm, creating a wall which was twice as thick as the 2.5 mm dialysis tubing. Under microscopic inspection, air bubbles were trapped between the wall layers. Even though this tubing was

dramatically different than the other diameters tested, it would still have only underestimated the true flow rate by approximately 11%.

To test the couplant material, the probe was placed around sections of dialysis tubing in vitro and intact arteries in vivo. Acoustic couplant was placed between the vessel and the transducers and held in place by a latex pocket. In the case of saline, the entire chamber or cavity holding the vessel/probe assembly was filled. Saline was found to be the best couplant in terms of signal strength. In addition, saline was also the easiest to use, in that it surrounded the probe body and required minimal vessel handling. The literature has suggested that Nalco 1181 was a suitable couplant material. In this investigation, it proved to be difficult to mix properly and difficult to remove trapped air even after spinning. Coagulated blood has been suggested as a couplant material for acute applications but the procedure necessitates spinning down the blood, removing the serum and carefully injecting the plasma into the probe window. Saline, therefore, is the preferred couplant for all acute application, whether in vitro or in vivo.

The vessel within the sensing element of the transit time probe was placed in 5 different positions and the probe calibrated for each position. When the vessel is to the left or right of the centre of the probe (positions 1,4), the signal is slightly smaller than for the centre position for the same rate of flow. This is probably due to edge effects stemming from the difficulties in constructing a plane wave from an ultrasonic crystal. The crystal produces a wave which is planar over 70% of the beam width. This error may be minimized by insuring that the vessel is placed in the approximate centre of the flow probe (position 2, 3, 5), or a probe should be selected which properly fits the vessel in question. For all further analysis, the probe should either fit snugly over the vessel without constriction, or if possible, be sutured or clamped in place. This will minimize the possibility of vessel movement within the probe and prevents the probe from twisting, a problem which is due to the stiff cables used to attach the probe to the transit time blood flow meter.

The vessel alignment was varied from minimum to maximum misalignment and the flow probe was calibrated in these positions. There was a significant difference between the min/max misalignment positions of the vessel. Referring to equation [19], θ and α are approximately 45° . The vessel is free to move by about $\pm 10^\circ$, corresponding to the maximum misalignment position. A 10° reduction or increase in θ corresponds to a 10° increase or decrease in α . There is less than a two percent difference in the theoretical output signal between the minimum and maximum misalignment positions. The data clearly contradict this position. The difference may be due to inhomogeneities in the crystal structures and the inability to construct a wide, homogeneous acoustic wave. If the angle of deviation is allowed to increase to $\pm 20^\circ$ then the theoretical prediction does account for the measured data. The problem associated with vessel alignment may be minimized by ensuring that the probe is placed in the minimum misalignment position and sutured or clamped in place.

The method of transit time flow measurement depends on the medium in two aspects, that is, the velocity of ultrasound in the medium and the ability of the medium to transmit ultrasound. The difference in the signal for blood and the signal for saline may be attributed to the difference in the velocity of sound for these media. In blood, the velocity of sound is 1565 m/s at 37°C . In saline at the same temperature the velocity of sound is 4% lower at 1500 m/s. There is a 6% difference in the output signal for these two media. This theoretical difference is confirmed by the probe manufacturers⁹⁷. Experimentally, the difference between the signal for blood/saline and saline was 10%. The discrepancy may be that the blood used in vitro was not fresh whole blood, but a mixture of red blood cells and saline commonly used in isolated organ research. The electrical properties of this blood substitute may match those of whole blood, but perhaps the acoustic properties are different. If the calibration of the perivascular probe in vitro with saline is compared to the calibration of the same probe in vivo, as in Fig. 32, the signal for saline is 12% lower than that for whole blood. For the cannulating probe the difference between saline and whole blood is only 8%, slightly less than the perivascular probe. The difference therefore cannot be entirely attributed to the acoustic properties of

the blood/saline mixture. The difference may be due to slight changes vessel position. The in vivo calibration using whole blood at 39.0°C will be used for both the perivascular and cannulating probes to assess the in vivo signal recorded by the flow meter.

The velocity of sound in the medium is a slowly varying function of temperature over the range of temperatures in this investigation. For blood, there is a 1.48 m/s/°C change in signal⁹⁸. The change for saline is approximately 0.3 m/s/°C. For a range of temperatures from 35 to 45°C this is a change of 15 m/s for blood and only a 3 m/s change for saline. The velocity of ultrasound in blood will vary from 1565 m/s to 1580 m/s. From the theory, this would result in less than a 2% change in reported flow for blood and less than 1% for saline. The experiments above were unable to detect any temperature dependence over this range for saline. The blood-saline mixture showed a 3% change in the recorded signal from 35 to 45°C. Since in vivo temperature changes are not expected to vary by more than $\pm 4^\circ\text{C}$, no corrections will be made to the in vivo signal. If the probes were to be used under hyperthermic or hypothermic conditions, then they would require calibration at these temperatures using whole blood.

The signal drift of the ultrasound probe around a section of dialysis tubing filled with 39.0°C isotonic saline was monitored over a period of eight hours. The data are shown in Fig. 38. After a period of one hour the average signal did not change. The error in this measurement is due to the resolution of the stripchart recorder and the pulsatile pump. The transit time ULBFPs should be allowed a period of one hour to warm up. After the initial warmup period, the drift of the signal is below the resolution of the measurement devices and can be ignored. The smallest change in flow which resulted in an observable change in the output signal was 2 ml/min.

5.3 Thermal Pulse Decay Probes

Before discussing the results of the experiments it is necessary to consider why the measured decay constant versus flow curves were fit with fourth order polynomials and

The downward trend in the decay constant also seen in the theoretical data is clear, but there is a levelling off of the curve for higher flow rates. The higher order fit was necessary to include the data in the high flow region. The difference between the measured and calculated data is most likely due to the assumptions of the model. It was assumed that the probe was a point source, but in fact was a sphere with approximately a 1 mm radius. The thermistor is a composite structure composed of the thermistor, a glass coating and a thin layer of epoxy, each with their own thermal properties which may be quite different from that of the surrounding tissue. The thermistor has long leads which may transport away a significant portion of the deposited power, this was not accounted for. To better analyze the discrepancy between the predicted and measured response voltage responses, a future study of the spherical source model⁴⁸ (SSM) may be profitable. The BHTE is known to have difficulties in calculating the temperature field in and around large vessels. In addition to considering improvements to the measurement circuit, other models, such as those recently reviewed by Kress and Roemer⁹⁹ should be examined.

The thermal pulse decay probe was placed in a T type connector. Degassed distilled water was perfused through the T at a constant temperature. To examine the probes response under steady flow conditions, a rotameter was used to control the volume rate of flow. Similarly, for pulsatile studies, a rotary pump was used to regulate the flow. In Fig. 39, the measured decay constant of probe #1 is shown for different flow rates for both steady and pulsatile flow. There is a 1% difference in the decay constant for the recorded calibration curves for pulsatile and steady flow. To the probe it appears that the perfusate is continually flowing around it. No correction will therefore be made for the type of flow that the probe is measuring.

With reference to Fig. 40, two type of perfusates or media were passed through the cannulating thermal probe. There is a marked difference of 11% between the calibration curves for water and that of saline. To determine what may be causing the difference, it is necessary to examine equation [32] (the temperature increase due to the heat pulse),

With reference to Fig. 40, two type of perfusates or media were passed through the cannulating thermal probe. There is a marked difference of 11% between the calibration curves for water and that of saline. To determine what may be causing the difference, it is necessary to examine equation [32] (the temperature increase due to the heat pulse), assuming that the measured voltage response is still determined by these factors even if the functional form is not exactly as expressed in this equation. If the power deposited in the thermistor bead and the heating time are the same for both types of media, the difference may be due to the thermal conductivities, specific heat and densities of these two media. The density, thermal conductivity and specific heat for water and saline each differ by 1%. The theoretical bridge voltage (equation [43]) across the bridge, for zero flow, may be determined for both media. The parameters used for this calculation are given in Table I. When this theoretical data is fit with a single exponential function it suggests that there is a small (1%) decrease in the decay constant for saline. The observed difference in the decay constants for saline and water is not due simply to the differences in the thermal properties of these two media. Further investigation is required to determine what caused the marked difference in these media. One possibility may be position in the cannulating probe, although care was taken to reproduce the positions of the probes and to ensure that the probe was not touching the side walls of the T connector. If the probe when used with saline had touched these wall then the heat pulse would have been dissipated faster shifting the curve towards lower decay constants. A bubble trap minimized the possibility of air surrounding the probe. If some air did surround the probe when water was used then heat would not have been dissipated as fast and the decay curve would be shifted up towards higher decay constants. The exact cause of the difference between decay constants is most likely due to systematic error and not a difference in the thermal properties of saline and water. This difference is very interesting, since it indicates that the probe is very sensitive to how it is used and positioned. It may be noted that this large difference was for probe number #2. The difference between the curves for probe #1 was slightly less at approximately 5%. This is one reason why probe number #1 was used for most of the investigations.

Fig. 54 The voltage measured across the bridge circuit is shown for several background temperatures. The upper and lower curves at each temperature represent 0 and 30 ml/min/g perfusion respectively.

Using equation [43] it is possible to characterize the behaviour of the TPD probe for different background temperatures. The voltage time response of the probe as determined by this equation is shown in Fig. 54. For the same perfusion there is less than a 1% variation in the decay constant of the fitted data with a change in background temperature from 35 to 45°C. The difference is attributed to being a property of the model and fit routine and does not arise from a physical difference due to temperature. In Fig. 42 and Fig. 43, the measured decay constants for these temperatures are shown for water and saline respectively. There is no significant trend in the decay constants as the background temperature of the medium increases, suggesting that it is not necessary to measure background temperature of the medium.

A cylindrical flow cell was constructed so that the thermal probe could be inserted into porous media perfused with saline. The temperature of the perfusate, 39.0°C, was held fixed with the use of the constant temperature reservoir and an in line heat exchanger.

The zero flow decay constants are shown, in Fig. 44, for zero flow at the same temperature for the porous media, as well as, several biological materials. The kidney, muscle, spleen and fat data were taken upon the death of an experimental animal at the end of the surgical protocol. Water had approximately the same zero flow decay constants as the sponge and kidney, muscle and spleen samples. Coarse and fine sand and isotonic saline were also similar. In Fig. 55, the measured decay constant for all these materials is plotted against their conductivities taken from various sources^{100,101,102}. There is a definite decrease in the decay constant as the thermal conductivity of the medium increases. This suggests that an improved TPD probe might be used to determine the thermal conductivity of an unknown medium. This would be a distinct characteristic of this method of flow measurement, since with a single series of measurements it is possible to not only measure flow but also the thermal conductivity of the tissue surrounding the probe.

Fig. 55 The measured decay constant of the TPD probe for different inorganic and organ materials versus their known thermal conductivities.

In Fig. 45, the decay constant of probe #1 is shown for the same total flow rates through the flow cell. The data are plotted against the distance of the probe from the centre of the cell for three porous media; sponge and coarse or fine sand. The exact effect of the porous medium on the velocity profile in the flow cell is not known, but it is assumed that some velocity gradient would exist. The flow at the side wall is assumed to be zero at the edge of the cell and maximal at the centre. For 1 cm about the centre of the flow cell the decay constant remains the same as the centre value for all three media. The decay constants for these media increase to its zero flow rate value over the next 0.5 cm (the edge of the cell). This suggests that the flow through the cell was non-uniform. This supports the assumption that there is zero flow at the edge of the cell and maximum flow at the centre. The TPD probe could be used to measure large flow rates associated with major arteries and with improvements in resolution may be able to measure the velocity distribution across the vessel. This would still be a crude estimate of the velocity distribution, since the probe has effective measurement volume of a sphere of 4 probe radii.

In order to further calibrate the thermal pulse decay probes against flows *in vivo*, isolated swine kidneys perfused with saline at 39.0°C were used. The flow, or perfusion in this case, was determined by the radioactive tracer microsphere technique. The thermal probes, inserted into swine kidneys, could not measure the low flow rates associated with intrarenal blood flow accurately. For the three kidneys tested, Fig. 47 clearly illustrates this fact. Although there is a definite decrease in the decay constant of the TPD probe for increasing perfusion values, the data is near the resolution of the probe. The resolution of the cannulating probe was approximately 10 ml/min. For a typical sample size of 10 g, this represents a resolution of about 1 ml/min/g. To accurately measure intrarenal blood flow a more sensitive version of the thermal pulse decay probe than that constructed in this investigation would be required.

The damage caused to the internal tissue structure due to the introduction of the flow probe is not known. The introduction of the probe will invariably cause some local

damage to the surrounding tissue, such as clots, and the flow in the vicinity of the probe may be compromised. For in vivo applications, the TPD probe must be biocompatible since the immune system would attempt to isolate it, depositing materials which adhere to the probe body and possibly altering the thermal properties of the probe. Isolated and in vivo kidneys may prove advantageous in the process of quantifying the effects of probe trauma and biocompatibility,

Several researchers have constructed TPD probes which are capable of measuring perfusion in tissue^{35,38,39,40}. These probes typically have accuracy to within $\pm 10\%$ in terms of calculating perfusion and thermal conductivity of the tissue. With further improvements the TPD probes used in this investigation may approach the accuracy obtained by these researchers. To construct a TPD probe which is sensitive to changes in intrarenal blood flow, several improvements to the present probe design would be required. Thermistors that are very sensitive to changes in temperature would also be required, since the low perfusion values associated with the flow within the kidney, implies that there is little difference between the temperature decay curves for 0 ml/min/g and a considerably higher flow rate of 3 ml/min/g. Microthermistors, with a probe diameter of less than 1 mm may be useful, since they would be a better approximation of a point source than the probes used in this investigation and hence the model describing the probes behaviour would be more applicable. Consistent with this approach, modelling these probes using the better approximations, such as the spherical source model would be an improvement. A model which included the finite volume of the probe would necessarily be a better estimate to thermistor/tissue environment. By increasing the power deposited to the bead or extending the heating time, a larger temperature/voltage increase occurs for a given flow rate, however these parameters are limited by the tolerance of the thermistor device and the trauma to the tissue associated with an increased thermal dose. More study would be required to determine the balance point between these competing factors. Probes which were more robust would also be an advantage since of the four probes constructed only one (Probe#1) survived to the end of the trials.

5.4 RTM Method

An autoperfused kidney was used to assess the trapping efficiency of the 15 μm diameter microspheres. The arterial and venous outflow of the kidney were sampled for 1 minute over the duration of the injection. No isotopes were found to be in the venous blood samples. This indicates that the trapping efficiency of the kidney using 15 μm diameter microspheres is essentially 100%. This is supported by the data from Capurro¹⁰³, et al.; who working with dogs, concluded that virtually no microspheres of this diameter would be lost provided that the animal is sacrificed less than 24 hours after the injections. It is, however, possible that no microspheres were detected because the venous sampling time was too short and additionally, the concentration of the microspheres in the venous blood would be below the resolution of the detector system in its present configuration and for a 10 min acquisition time. If this time was extended to obtain sufficient counts, perhaps it would reveal some activity in the venous sample.

To examine the assumption that the microspheres were well mixed with the blood, the flow to the left and right kidney pair was analyzed. The data from the first microsphere injection from several experiments were used since the left kidney at this point had undergone little or no surgical manipulation. A measure of good mixing is that the left and right kidneys flows should be equivalent. The data in Fig. 52 suggest that this is true, hence the microspheres may be said to be sufficiently mixed with the blood when injected into the left ventricle of the heart.

The microspheres appeared to have been trapped by the organ and were well mixed with the blood. In addition, in the isolated organ experiments the addition of microspheres did not increase vascular resistance, indicating that no significant fraction of the capillary bed was blocked by the presence of the spheres. This demonstrates that the 15 μm diameter microspheres are appropriate for studies of renal blood flow in pigs and that the number of microspheres used for an injection was appropriate. This is supported by Phibbs¹⁰⁴

et al who concluded that, while smaller diameter microspheres have better blood like properties, 15 μm diameter spheres were trapped more efficiently than 7-10 μm spheres.

An isolated saline perfused kidney and a precalibrated rotary pump were used to assess the microsphere method in vitro. Microspheres were injected into the kidney at a rate of 1 ml/min six times over a period of approximately 2 hours for flows ranging from 0 to 150 ml/min. The flow as determined by the RTM method was plotted against the flow from the pulsatile pump and fit with a linear equation, as shown in Fig. 49. For this data set it would appear that the microsphere method tends to overestimate the total flow through the organ at higher rates of flow. This overestimation may be due to the source size of the tissue samples. In the reference samples the microspheres tend to concentrate near the bottom of the sample along with the red blood cells. In contrast, the tissue samples trap the microspheres and have a greater source size. The tissue samples therefore act as extended, as opposed to point sources of radiation. If the exact tissue detector configuration is known then this effect can be corrected. A more practical method would be to homogenize the tissue and vortex the solution, concentrating the microspheres at a point in the test tube¹⁰⁵. The drawback to this method is the increased time; e.g., several days, required to prepare the samples. In addition, to avoid the loss of spheres from manual homogenization, chemical homogenization of the tissue is required. Chemicals which are capable of quickly breaking down the structure of the tissue sample are often also capable of dissolving the microspheres and causing unnecessary isotope leaching. The leaching problem may be eliminated by using glass conical test tubes instead of polypropylene tubes, but this adds to the cost of the method.

To determine if the microspheres were well mixed, the microsphere distribution achieved in the in vivo kidneys was compared to the distribution found in the in vitro kidneys. The perfusion data for the in vitro and in vivo kidneys is illustrated in Fig. 50. The profiles from the superior to inferior sides of the kidneys realized in vitro are qualitatively similar to those in vivo. The seemingly lower perfusion values of the in vitro data are due to the higher flow rates associated with the native kidneys. The microspheres are

well mixed in vivo since left and right kidney flows are equal. The similarity in the perfusion profiles in vitro and in vivo therefore implies that the microspheres are also well mixed in vitro, and distribute themselves throughout the kidney in the same manner as the perfusate. The isolated organ is an ideal way to calibrate the microsphere method in vitro. The in vitro calibrations will be used to determine the flow from the in vivo microsphere data.

In several in vivo experiments, 30 to 60 s after the first microsphere injection, the animal experienced abnormalities in heart rate accompanied by a decrease in the left ventricle pressure. It has been suggested that this effect is due to the presence of Tween in the microsphere solution¹⁰⁶. To determine if the effect was due to the Tween, different concentrations of Tween were injected, without microspheres on three occasions. No effect was seen but the sample size was low. More studies would be required to determine if the physiological stresses observed after the first microsphere injection were caused by the microspheres or the presence of Tween. In any case, the microspheres have usually reached and been subsequently trapped by the organ being studied before this effect occurs, so in theory, the data should still be acceptable. In this investigation, data accompanied by this effect were not used since their validity is questionable.

For long term studies (greater than 24hrs) it may be necessary to determine if the injected microspheres have remain trapped in the tissue. It is possible that dilation of the capillaries may result in the surrendering of microspheres to the blood stream once again. If so they are likely trapped by another organ. This would result in an underestimate of the tissue perfusion at the time of injection.

To determine if the microspheres accurately distribute themselves in the same manner as whole blood, another technique, such as a thermal pulse decay probe or the use of tritiated water⁵⁵ may be of used to assess the local perfusion throughout the organ. In this investigation the microspheres are assumed to distribute themselves through the kidney in the same manner as whole blood.

6. Conclusions and Comparison of Blood Flow Techniques

6.1 Electromagnetic Flow Probes

Electromagnetic flow probes are stable accurate devices capable of measuring blood flow independent of the type of flow or the medium temperature over the range investigated. From section 2.1, a linear response of the probe versus flow was predicted and clearly shown in the experiments. Cannulating electromagnetic probes are useful for monitoring extracorporeal blood flow, but are often impractical in vivo. Perivascular EMBFPs are suitable for in vivo use, but the versatility of a given probe is severely limited since the probes are vessel diameter dependent and require direct electrical contact with the vessel wall. In addition, acute application of the probes requires that the vessel be slightly constricted which may result in physiological changes, both regionally and systemically. This limits the number of locations in which the EMBFPs may be used. The velocity profile of the blood in the constriction is altered, however the device measures total volume flow and not velocity profile. The difference in the conductivities between saline and blood indicates that the calibration of the probes in vitro with saline introduces an error of 4% if the probes are then used in vivo to measure blood flow. Probes should be calibrated with fresh whole blood, not saline as the literature suggests. To reduce the effect of signal drift, probes should be allowed to warm up for a period of at least one hour. In addition, if the probes are to be used to monitor blood flow under hyperthermic or hypothermic conditions, then calibration of the probes at extreme temperatures would be required.

6.2 Transit Time Probe Conclusions

Transit time ultrasound probes are stable devices which are able to resolve small changes in flow. Both cannulating and perivascular type probes are capable of measuring blood flow independent of the type of flow and the perfusate temperature. Perivascular probes

are also independent of the diameter of the vessel placed in the sensing volume of the probe. For acute applications, isotonic saline used as an acoustic couplant results in a better signal strength than the other couplants tested. Dialysis tubing would appear to be a vessel-equivalent material and is appropriate for the in vitro calibration of the perivascular probes. Placement of the vessel centrally in the probe volume is necessary in order to measure the flow accurately. Although less sensitive to angle misalignment than previous versions, care must be taken that the transit time probes are properly aligned with the vessel. Transit time probes are not tight fitting, do not constrict the vessel, but do require the use of an acoustic couplant medium. The experiments indicate that best couplant medium to use for acute application is isotonic saline. Transit time ultrasound probes should be capable of measuring net flow over bifurcated vessels. To properly calibrate the ULBFPs, in vivo calibration utilizing whole blood is required, since in vitro calibration using saline or a blood cell saline mixture results in an error on the order of 10% in the measured output signal. The probes should be allowed to warmup for a period of one hour prior to acquisition of data.

Future experiments with the transit time ultrasound probes should include the investigation of veins as well as arteries. The effect of changes in temperature using whole blood must be examined if the probes are to be used under hyperthermic conditions where locally temperature may exceed 47°C. It may also be prudent to measure the actual velocity profile of the types of flow used in this experiment to conclusively determine if the signal is independent or dependent on the flow profile.

6.3 TPD Conclusions

The thermal pulse decay probes used in this investigation exhibit the potential for measuring total renal blood flow. The T-type cannulating TPD probe was capable of measuring flow rates up to 120 ml/min with an accuracy of $\pm 15\%$. However, as the difference in the curves for saline and water illustrate, the device is particularly sensitive to positioning within the T type connector. This type of probe would have limited use

since delicate surgical techniques are required to attach the probe in series with the kidney. In addition, calibration in vitro using whole blood is required to investigate the probes response and therefore better predict its in vivo performance. In the in vitro tests, the cannulating thermal probes were found to be independent of the type of flow (steady or pulsatile). The probes were only slightly dependent on the temperature of the perfusate. Using an isolated saline perfused kidney and the microsphere method it was determined that the thermal pulse decay probe used in this investigation may be used for the study of intrarenal blood flow but the poor resolution of this design prohibits accurate measurements.

Several modifications were proposed to increase the accuracy of the present TPD probe design used in this investigation, including smaller and more sensitive (in the 35-45°C range) thermistors and modelling the thermistor as a composite finite volume device. If these probes are eventually to be used in vivo, care must be taken to ensure that the position of the probe is maintained through the experiment. The probes are sensitive to the thermal conductivity of the surrounding media. If cold bolus or dyes are injected or the tissue temperature is stressed beyond the range tested; e.g. hyper/hypothermia) then the appropriate calibrations would be required. If the intention is to use the TPD probes in numerous organs, then they should also be calibrated in a number of organs using the same phantom setup discussed in this report.

6.4 RTM Conclusions

The radioactive tracer microsphere method is a technique for determining the total flow through an organ or several organs at a particular point in time. In vivo experiments performed on isolated saline perfused kidneys indicate that the technique overestimates the total flow through the organ, but it is possible that this may be overcome by accounting for source size or by tissue homogenization. In vivo experiments demonstrate that the microspheres are well mixed with the blood after injection and distributed throughout the organ of study. It is assumed that this distribution is identical to the

distribution of whole blood flow through the organ. It is important to monitor the ECG and relevant pressures during and shortly after an injection to determine if the injection caused any adverse effects. The technique described here requires several modifications for use in humans. Specifically, starch microspheres with low activities and short half lives should be used and the activity determined with the use of a gamma camera¹⁰⁶.

The main difficulties of the RTM technique are that it does not record flow continuously and is procedurally complex. Its potential lies in the possibility of determining local perfusion throughout several organs simultaneously. In theory, the technique does not depend on temperature and thus may be quite useful in determining perfusion during hyper/hypothermic treatments, and particularly those involving systemic treatments.

6.5 Comparison of the Four Techniques

The principle of operation of the four methods used for measuring total renal blood flow in this investigation have been examined in the preceding chapters. The methods were calibrated in vitro and in vivo under various experimental conditions. It remains to compare the techniques to determine which one, or ones, is most suitable for the purpose of measuring the total flow through an organ. Several criteria are used to determine the suitability of the probes. The best probe would have the highest accuracy. This is the chief consideration, since, as seen in chapter 1, accurate blood flow measurements are required for theoretical models of temperature distribution in tissues, the efficient delivery of chemotherapeutic agents and the knowledge of the transport of blood gases, waste and nutrient material. Most importantly, however, is an accurate knowledge of blood flow and its effect on the tissue or tumour environment, particularly with respect to hyperthermia treatments.

The second consideration when determining a probe's suitability is its stability. The flow signal produced by the ideal probe should not change over the measurement interval or with other interventions such as a change in the perfusate temperature. If this cannot be

accomplished, then the manner in which the probe's signal changes under these stresses should be well understood and accounted for. Versatility is another important factor when comparing different flow probes. The ideal probe would measure flow through different diameter vessels in a variety of sites within the animal and have the capability to measure local flow as well as the total flow through the organ.

Although accuracy, stability and versatility are the main factors when considering a probe, the initial and subsequent application costs, as well as, the ease of operation of the probe may also be considered. The ability to use the probe in chronic applications and in humans is another factor.

6.5.1 Comparison of In Vitro and In Vivo Flow/Perfusion Measurements

The comparison of the flow/perfusion measured by the four techniques was one of the main objectives of this research. There are two basic comparisons which may be made and these are based on the two types of flow that were examined: 1) bulk or total flow through a vessel or organ and 2) the local perfusive flow. The latter comparison is necessarily limited to the tracer microsphere technique and the thermal pulse decay probes.

6.5.2 Total Flow through a Vessel or Organ

All of the techniques examined are capable of measuring the total flow rate of a perfusate through an organ. The comparison of the four techniques is shown in Fig. 55. The pulsatile flow of 39.0°C isotonic saline for the various devices is plotted against the flow rate of the pump. The device flow rates were determined by using the calibration equations for pulsatile flow of saline at 39°C for each probe respectively.

The data may be fit with the linear equations $y=mx+b$. The resulting fits for the four devices are shown in Table III. A slope of one implies that the device was recording the true flow rate determined by the pulsatile pump. The ultrasound probe had the slope which was closest to one. The thermal probe had the worst slope and highest zero flow

Fig. 56 Comparison of pulsatile saline flow measured by the RTM technique, perivascular ultrasonic and cannulating TPD and electromagnetic probes. The comparison of the using whole blood for the EM and UL probes is also shown.

offset. In order of accuracy of the probe ranking is: the ultrasound probe, the electromagnetic probe, the microsphere technique and the thermal pulse decay probes. Table III also indicates that the thermal pulse decay probes are inadequate for measuring total flow and in their present design should not be used for this purpose.

Also shown in Table III are the fit parameters for the comparison of the transit time ultrasound probe and the cannulating electromagnetic probe for whole blood. The data for this comparison are shown in Fig. 56. and are from an in vivo experiment using an animal prepared as per Appendix D. The left femoral artery and vein were isolated and catheterized. The pulsatile pump was used to draw blood from the artery, through the cannulating probe and returned to the animal through the femoral vein. The 4 mm ultrasound probe was placed around the artery, taking care that the cannula in the artery was not in the sensing element of the probe. By altering the flow with the pump, both the ultrasound and electromagnetic probes would measure the same volume flow of blood. As can be seen in Fig. 56, the slope for the ultrasound probe is closer to one and the fit has a smaller zero offset than the electromagnetic probe. The two curves agree and indicate that they are both capable of measuring blood flow accurately. It is clear from Table III that in terms of measuring total flow rates both in vivo and in vitro, the ultrasonic probes are superior.

6.5.3 Comparison of Perfusion In Situ

Using the calibration of the thermal pulse decay probes for perfusion in isolated saline perfused kidneys it is possible to compare the perfusion measured by the microsphere technique and the thermal probe in situ. Implicit in this analysis is the assumption that the RTM method accurately describes the local perfusion of blood through the organ. Three female swine were prepared as per Appendix A and the left kidney was exposed. A thermal pulse decay probes was inserted 1 cm into the inferior

Fig. 57 The comparison of the flow of whole blood controlled by the pulsatile pump measured by the 4 mm perivascular and cannulating ultrasonic flow probes.

Fig. 58 Comparison of blood perfusion measured by TPD probe #1 and the RTM technique in native kidneys.

Device	Slope	Offset (ml/min)
Electromagnetic	1.13 ± 0.10	$-(8 \pm 8)$
Ultrasonic	1.04 ± 0.08	$-(9 \pm 6)$
Microsphere	$.965 \pm 0.12$	4 ± 10
Thermal Probe	$.579 \pm 0.16$	12 ± 15
Electromagnetic Whole Blood	$.927 \pm 0.11$	4 ± 9
Ultrasonic Whole Blood	$.987 \pm 0.08$	3 ± 6

Table III The first four entries show the linear fit equations for the four devices measuring saline flow in vitro, through vessels or isolated organs. The last two entries in the table describe the fit equations for the comparison of the ultrasonic and electromagnetic probes using whole blood.

ventral surface using a small needle to create the initial entrance aperture. The thermal pulse decay probe was monitored during the microsphere injection times. Six different isotopes were injected over a period of 1 to 2 hours.

The data from these experiments are shown in Fig. 57. Fitting the data with a linear function, the fit equation becomes, $y = (4.43 \pm 1.39)x - (6 \pm 2)(\text{ml/min/g})$. There is an extremely low zero offset. The two devices do not agree on the measured perfusion. This fit is important, since it indicates that there is relationship between the perfusion measured by the two devices. As the perfusion according to the RTM method increase, there is a noticeable increase in the tissue perfusion measured by the TPD probe. The data are encouraging and suggest that with some improvements the thermal pulse decay probe may be capable of accurately determining local perfusion values. However, until a more accurate TPD probe is developed, the microsphere technique is preferred.

6.5.4 Versatility and Conditions of Use

The ultrasound probes were by far the most versatile of all the probes tested. The perivascular transit time probe could measure flow through vessels of different diameter, and although not tested in vivo, the ability to measure net flow through bifurcated vessels and vessel pairs was demonstrated in vitro. This is a major advantage of these probes since none of the other methods tested to measure total flow could repeat this task. The thermal probes can measure flow in only a single artery and since no trapping occurs in the vessel, the microsphere technique cannot be used to determine the flow through vessel pairs or branches. Since the signal produced by the electromagnetic flowmeter is dependent on the properties of the vessel wall such as electrical conductivity and diameter, it is unlikely that the electromagnetic probe would be able to accurately measure net flow through the vessels in question. More study is required, but it appears that only the ultrasound probe could be used to measure blood flow over pairs of arteries. The presence of artery-pairs supplying the kidney was frequently observed in these investigations and the ability to measure this sort of flow with one probe would be an asset.

Another distinct advantage of the perivascular ultrasound probes is that they do not require direct contact with the vessel. The electromagnetic probes constrict the vessel causing adverse physiological effects and the TPD probes would require that the vessel be ligated or a purse string suture be used. The microsphere method does not affect the vessels around the organ of study, but cannulation of the left ventricle and the necessity of a reference organ make it a more invasive procedure on the whole. When it is important to manipulate the organ of study as little as possible, as is often the case, the ultrasound probes should be used since minimize the amount of vessel handling.

The microsphere method has the advantage that it can be used to determine the local tissue perfusion throughout the organ. The thermal pulse decay probes are also capable of this but in their present configuration they are not nearly as accurate. The ultrasound and electromagnetic probes are strictly not able to measure intraorgan blood perfusion. The microsphere technique also has the novel advantage that it can measure blood flow through several organs simultaneously and has no rival in this area. The other probes measure flow through a single (or possibly multiple) vessel or organ. When local perfusion or flow to several organs is required simultaneously the, RTM method is the method of choice.

A disadvantage of the RTM method is its inability to continually monitor the flow rate through the organs. The RTM technique is capable of providing only snapshots of the flow in time and the number of these snapshots is limited by the number of isotopes available, the detector resolution and the number of microspheres which may ultimately be injected. The present method requires excising the tissue for counting and is general not performed on humans, since special procedures, such as low activity starch sources are required.

As far as media temperature effects are concerned the probes may be used with confidence in the range 35-45°C. If the medium temperature deviates from these

calibration temperatures, such as in hypo/hyperthermia treatment then the probe may be recording flow erroneously.

The thermal pulse decay, ultrasonic and electromagnetic probes have a functional dependence of the perfusate used. Since these probes are ultimately for use in humans who are ill, attention must be made to the condition of the patient and the site of flow studies. The illness may cause unexpected alterations in thermal and electrical conductivity as well as in the velocity of ultrasound through the medium. The site of use is also a factor which must be considered. Consider the following example: Ultrasound flow probes require a couplant material and the best couplant was determined to be saline. In chest operations the body naturally forms a cavity to contain the acoustic couplant. If the site of flow measurement is an artery on the leg, this becomes a major problem. In this case, an electromagnetic probe may be more appropriate, since it only requires contact with the vessel and not a couplant media.

Another consideration is the effect of vessel movement. Both the ultrasound and electromagnetic probes are susceptible to alterations in vessel movement. For strongly pulsing vessels, the electromagnetic probe's electrodes lose contact with the vessel wall and subsequently experience a dead time until the vessel again makes contact. The flow signal for ultrasound probes was shown to vary with vessel orientation and position. The probe must therefore be secured in place to avoid this eventuality. These factors limit the use of the probes since they should not be used in the vicinity of large strongly pulsating vessels.

These problem illustrates the fact the there is no one ideal probe. Each case must be considered individually. However if there is a choice of probes the ultrasound probe should be used because of it superior accuracy and stability.

Similar ultrasound, electromagnetic and thermal pulse decay probes have been used by other investigators in chronic experiments on sheep⁵², pigs^{50,51} and cattle⁴⁹, as well as a

number of other experimental animals^{74,106}. The ultrasound probes prove to be the most stable of these probes, requiring the least zero offset verification and recalibration. The microsphere method can be used in chronic studies¹⁰⁷, provided that the duration of the experiment does not allow for the isotopes to decay to the point where they are not detectable by the gamma counter. Again, the inability of the RTM technique to continuously measure the volume flow of blood is a distinctive disadvantage of this method. All of the devices have the ability to be used in humans. Which probe or technique is appropriate again depends on the type of disease or surgical procedure. A thermal probe may be easily inserted into an arm or leg to measure perfusion, the ultrasonic and electromagnetic probes are well suited to studies in the body cavity such as organ transplantation and the microsphere method is particularly suitable for studies of blood flow in the brain¹¹². If microspheres are to be used in humans care must be taken to minimize patient risk by using short lived sources.

6.5.5 Initial and Subsequent Cost

Cost is the least important consideration from a scientific point of view, but cost effective probes should be selected over less accurate more expensive techniques. Again the ultrasound probes proved to be probe of choice, since they were the most accurate and least expensive. The initial cost of the flow meter is \$10K, and probes range from \$300 to \$500, with little subsequent upkeep. The cost of the electromagnetic flow meter is approximately the same as the ultrasound meter, but the probes are twice the price. This is significant since many probes would be required to measure different diameter vessels, in sharp contrast with the 2 or 3 probes needed to effectively operate the ultrasound meter. During the experiments, the stiff cables of the electromagnetic flow probe broke. These cables are not user serviceable and the new cables cost \$1000. The ultrasound cables are user serviceable and in any case may be replaced for a tenth of the cost. The cost of the thermal probes is on the order of \$10000 to \$15000, including the data acquisition language, devices and the computer. The probes themselves were built for approximately \$80 each, but as mentioned previously, were extremely fragile. The microsphere method is prohibitively expensive. The cost of each microsphere injection

is approximately \$100 and it is not reusable. The major initial cost of the system is the gamma counter, at approximately \$50000.

6.6 Summary and Future Prospects

The initial objectives set out in the introduction have been met. The principle of operation for each of the devices or techniques was developed from a few basic assumptions. The measurement artifact associated with the probes was studied under various conditions which are dictated by the underlying principles of the technique. For instance, media with different, densities, thermal, electrical and acoustical properties were used. The probes were subsequently calibrated so that the effects of the different physical interventions could be accounted for. Lastly, the flow measured by all of these probes was compared, as was there accuracy, versatility and ease of use.

In Table IV the many features of the four blood flow techniques are compared. It is clear that the transit time ultrasound probes are the most accurate and stable of the blood flow probes examined in this report. The transit time probes proved to be significantly more accurate than either the thermal pulse decay probe or the RTM technique. The versatility and ease of application of the ultrasound probe is unparalleled. These probes are capable of measuring flow over different diameter vessels, whereas the electromagnetic probes are designed to fit over a single diameter vessel. The electromagnetic probes are incapable of measuring the flow over bifurcations. In one of the experiments it was illustrated that the ultrasound probe was able to measure the total flow through two vessels placed within it sensing window for both counter-current and parallel flow.

The thermal pulse decay probe used in situ cannot measure total renal blood flow, in order to do so, a cannulating probe would be required. This is both impractical and unnecessary since the comparison of the cannulating ultrasound and thermal pulse decay probes in vitro clearly indicate that the ultrasound probes are vastly superior.

While the thermal pulse decay probes are capable of measuring the flow distribution through the kidney continuously, many probes at different depths would be required, a condition which is both surgically and technically difficult. The RTM technique is a much more reliable and accurate method of measuring total renal blood flow. Its chief disadvantage is that it may be used at only discrete points in time.

Where possible, transit time probes should be used to measure total organ blood flow. Care must be taken to ensure proper position and orientation of the probe and vessel, if the desired accuracy is to be attained.

If knowledge of the local tissue perfusion is required, then the microsphere method should be used. Although the technique was never calibrated for local perfusion, it calculates total organ flow accurately and the perfusion profile across the organ is repeatable, suggesting that the perfusion values may also be correct.

Several further experiments have been suggested throughout this report. The most important of these deal with the thermal pulse decay probes. It is essential that a reliable probe be constructed and other thermal models be investigated. A probe which can accurately determine perfusion and thermal conductivity of tissue samples continuously for a fraction of the cost of the microsphere method is desirable. The microsphere method must be validated in other organs. The left and right hemispheres of the brain may be examined to further determine if the microspheres are well mixed. The local perfusion values determined by the technique are not calibrated. The use of magnetic resonance imaging may be used to determine perfusion in the organ and perhaps establish or refute the validity of the method¹¹². Both the electromagnetic and ultrasonic flow probes should be tested for veins as well as arteries. This would increase the number of sites where these probes may be used by approximately a factor of 2. The study could also be expanded to include other methods of flow measurement such as transcutaneous doppler ultrasound. Future experiments should endeavour to complete these tasks.

	Ultrasound	Electromagnetic	Thermal Pulse Decay	RTM Technique
Accuracy	5-6%	5-10%	40%	10%
Stability	1%	2%	10%	10%?
Resolution	2 ml/min	5 ml/min	10 ml/min 1 ml/min/g	not tested
Different Vessels	Yes	No	No	No
Local Flow	No	No	Yes	Yes
Use in humans	Yes	Yes	Yes	Yes
Animal Sacrifice Required	No	No	No	Yes (Except humans)
Interference with Vessel	Small	Constricts	Ligation Required	Non Local
Initial Cost (\$)	10 000	10 000	15 000	50 000
Subsequent Cost	Low	Moderate	Low	High

Table IV Comparison of the features of the four blood flow probes used in this investigation.

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Appendix A

The potentials V_m and V_w may be found by solving equation [6]. It is assumed that the velocity of the liquid has a component only in the axial direction and that no angular gradient exists. If the magnetic field is parallel to the y axis, then,

$$\text{In the medium: } \nabla^2 V_m = -B \frac{\partial v}{\partial r} \cos \varphi \quad [A1]$$

$$\text{In the wall: } \nabla^2 V_w = 0$$

where φ is the angle with respect to the electrode axis as shown in Fig. 1.

Equations [A1] can be integrated in a straight forward manner. The rotational symmetry of the problem dictates that the solutions are of the form,

$$\begin{aligned} V_m &= W_m(r) \cos \varphi & 0 \leq r \leq a \\ V_w &= W_w(r) \cos \varphi & a \leq r \leq A \end{aligned} \quad [A2]$$

where the W functions are defined as the radial component of potentials.

Substitution of [A2] into [A1] results in the following differential equations,

$$\begin{aligned} \frac{\partial}{\partial r} \left(\frac{W_m}{r} \right) + \frac{\partial^2 W_m}{\partial r^2} &= -B \frac{\partial v}{\partial r} \\ \frac{\partial}{\partial r} \left(\frac{W_w}{r} \right) + \frac{\partial^2 W_w}{\partial r^2} &= 0 \end{aligned} \quad [A3]$$

The solutions to [A3] in the absence of boundary conditions are,

$$\begin{aligned}
 W_m &= -B \left[Q \frac{1}{2\pi r} + \frac{K_1}{2} r + \frac{K_2}{r} \right] \\
 W_w &= \frac{K_3}{2} r + \frac{K_4}{r}
 \end{aligned}
 \tag{A4}$$

where the K_i 's are constants of integration and Q , the volume flowrate, can be defined as,

$$Q = 2\pi \int_0^r r v dr \tag{A5}$$

For the problem of interest, the appropriate boundary conditions are:

$$\begin{aligned}
 A) \quad & V_m(0, \varphi) < \infty \\
 B) \quad & \frac{\partial V_w}{\partial r}(A, \varphi) = 0 \\
 C) \quad & V_m(a, \varphi) = V_w(a, \varphi) \\
 D) \quad & j_m(a, \varphi)|_{rad} = j_w(a, \varphi)|_{rad} \\
 E) \quad & \frac{\partial V_m(a, \varphi)}{\partial r} \sigma_m = \frac{\partial V_w(a, \varphi)}{\partial r} \sigma_w
 \end{aligned}
 \tag{A6}$$

Condition A implies that the potential is finite at the origin. Condition B implies that the insulating outer layer causes the radial current to be zero at the exterior boundary. Conditions C and D are expressions for the continuity of the potential and radial currents at the wall/medium interface respectively. Condition E states that angular currents at an interface compare inversely as the conductivities of the two media. Applying these boundary conditions, yields the potential in the medium¹⁴,

$$V_m = -\frac{B}{2} [\bar{v}(a) \cdot M + \bar{v}(r)] \cos \varphi \tag{A7}$$

where the constant M may be defined as,

$$M = \frac{\frac{\sigma_m}{\sigma_w} \left(1 + \frac{a^2}{A^2}\right) - \left(1 - \frac{a^2}{A^2}\right)}{\frac{\sigma_m}{\sigma_w} \left(1 + \frac{a^2}{A^2}\right) + \left(1 - \frac{a^2}{A^2}\right)} \quad [\text{A8}]$$

and

$$\bar{v}(r) = \frac{Q}{\pi r^2} \quad [\text{A9}]$$

In the wall,

$$V_w = -\frac{B}{2} a \bar{v}(a) N \left(\frac{r}{A} + \frac{A}{r} \right) \cos \phi \quad [\text{A10}]$$

where the factor N involving the wall and medium properties can be expressed as,

$$N = \frac{2 \frac{\sigma_m}{\sigma_w} \frac{a}{A}}{\frac{\sigma_m}{\sigma_w} \left(1 + \frac{a^2}{A^2}\right) + \left(1 - \frac{a^2}{A^2}\right)} \quad [\text{A11}]$$

The EMBFP has two diametrically opposed electrodes which measure the potential across the vessel at $r=A$ and $\phi=0,180$. The potential between the electrodes is,

$$V = 2B r \bar{v}(r) N \quad [\text{A12}]$$

The average velocity may be expressed as the volume rate of flow, Q , divided by the cross-sectional area of the tube, πa^2 . The volume rate of flow or simply the flow is the volume of medium passing through some volume per unit of time. In terms of voltage, the flow Q can be written as,

$$Q = \frac{-V \pi a}{2BN} \quad [\text{A13}]$$

Appendix B

In order to determine the signal for a wide beam, equation [14] must be integrated over the full transmitter-receiver area A_{tr} . This area is assumed to be a rectangle of length h and width w as shown in Fig. B1. The value of U_0 is assumed to be constant over this area.

The area seen by the wide beam includes the entire vessel cross section of area A_v , as well as some region external to the vessel of area A_e . For this case, the signal can be written as,

$$U = U_0 \left(\int_{A_e} \sin[\omega(t-T)] dydz + \int_{A_v} \sin[\omega(t-T)] dydz \right) \quad [B1]$$

In the region external to the vessel, $v_x=0$ and from equation [13], $T=L/c$. Therefore equation [B1] can be written as,

$$U = U_0 \left(A_e \sin\left[\omega\left(t-\frac{L}{c}\right)\right] + \int_{A_v} \sin[\omega(t-T)] dydz \right) \quad [B2]$$

The second integral term in equation [B2] may be expanded such that

$$\int_{A_v} \sin[\omega(t-T)] dydz = \int_{A_v} \sin\left[\omega\left(t-\frac{L}{c}\right)\right] \cos[\omega T_x] dydz \pm \int_{A_v} \sin[\omega T_x] \cos\left[\omega\left(t-\frac{L}{c}\right)\right] dydz \quad [B3]$$

For the transducer in question, the maximum crystal frequency is 1.5 MHz, L is of the order 0.01m, v is a maximum of 0.01m/s and c is approximately 1500m/s. In this case, ωT_x is less than 4×10^{-4} and hence $\sin \omega T_x \approx \omega T_x$ and $\cos \omega T_x \approx 1$. Then equation [B3] can be written as,

$$\int_{A_v} \sin[\omega(t-T)] dydz = \sin\left[\omega\left(t-\frac{L}{c}\right)\right] \int_{A_v} dydz \pm \cos\left[\omega\left(t-\frac{L}{c}\right)\right] \int_{A_v} \omega T_x dydz \quad [B4]$$

or,

$$\int_{A_v} \sin[\omega(t-T)] dy dz = A_v \sin\left[\omega\left(t - \frac{L}{c}\right)\right] \pm \cos\left[\omega\left(t - \frac{L}{c}\right)\right] \int_{A_v} \omega T_x dy dz \quad \begin{matrix} \text{B2} \\ \text{[B5]} \end{matrix}$$

and hence,

$$U = U_0 \left((A_v + A_r) \sin\left[\omega\left(t - \frac{L}{c}\right)\right] \pm \cos\left[\omega\left(t - \frac{L}{c}\right)\right] \int_{A_v} \omega T_x dy dz \right) \quad \text{[B6]}$$

where $A_r + A_v$ is the total area (A_{tr}) seen by the transmitter-receiver.

Substituting equation [13] into equation [B6] yields an expression for U in terms of the integral over the intersection of the flow and the acoustic beam such that,

$$U = U_0 \left(A_{tr} \sin\left[\omega\left(t - \frac{L}{c}\right)\right] \pm \cos\left[\omega\left(t - \frac{L}{c}\right)\right] \frac{\omega}{c^2} \int_{V_p} v_x dx dy dz \right) \quad \text{[B7]}$$

The velocity profile of the fluid in the vessel is assumed to have an arbitrary profile, then the integral in equation [B7]. The volume rate of flow, Q , is in general given as:

$$Q = \int_A w dA \quad \text{[B8]}$$

integration over the width of the beam, gives,

$$wQ = \int_0^w \int_A w dA dw \quad \text{[B9]}$$

The double integral may be combined to a single integral over the volume of the beam. To cast the integral in terms of the present geometry, the following transformation must be performed: $w \rightarrow w/\sin\theta$ and $v \rightarrow v_x/\cos\theta$,

$$\frac{w}{\sin\theta} Q = \int_{V_p} \frac{v_x}{\cos\theta} dx dy dz \quad \text{[B10]}$$

Rearranging and substituting equation [B10] into equation [B7], then for any given velocity profile,

Fig. B1 Geometry of wide beam illumination of vessel with a rectangular beam of area wh .

$$U = U_o \left(A_o \sin \left[\omega \left(t - \frac{L}{c} \right) \right] \pm wQ \frac{\omega}{c^2} \cot \theta \cos \left[\omega \left(t - \frac{L}{c} \right) \right] \right) \quad [\text{B11}]$$

Forming the difference in the upstream and downstream pulses, eliminates the first term and yields:

$$\Delta U = 2U_o Q w \frac{\omega}{c^2} \cot \theta \cos \left[\omega \left(t - \frac{L}{c} \right) \right] \quad [\text{B12}]$$

The result is a signal which is directly proportional to the volume rate of flow Q and the angle between the beam and the flow. The output signal is a direct function of flow and depends on the angle between the vessel and the beam. It is also a function of the specific probe geometry including parameters such as path length and crystal frequency.

Appendix C

Data Acquisition Program for the Thermal Pulse Decay Probe

```
\ THERMIST.PRG
\
\ Program by Andrew Weber
\
\ This program reads the output voltage across a thermistor/resistor bridge
\ circuit. The bridge consists of a thermistor and three resistors
\ Current is forced through the thermistor, raising the temperature of the
\ thermistor above ambient. The applied heating voltage is then removed and
\ the thermistor is allowed to return to ambient temperature.
\
\
: It nop ;    \ A word to forget easily
\
\ Load necessary words to fit data
\
Load.overlay Matfit.sov
\
\ *****ACTIVATE DAQ KIETHLY*****
\
Series.500
\
\ *****DECLARATION OF VARIABLES*****
\
Real Scalar Vheater          \ Voltage applied to the heating
                             \ branch of the circuit
\
Real Scalar Vapplied         \ Voltage applied to the bridge,
                             \ it should not exceed 2.0 Volts
\
Integer Dim[ 106 ] Array V1aoutput \ Output Voltage of Thermistor
                                     \ during heating
Integer Dim[ 151 ] Array V1boutput \ Output Voltage of Thermistor
                                     \ after heating
\
Real Scalar V1average        \ Average Voltage of Thermistor
\
Real Dim[ 150 ] Array Temp1.curve \ Voltage curve for Thermistor
Real Dim[ 150 ] Array Time1
\
Real Scalar Amplitude        \ Exponential Fit Parameters
Real Scalar Bexp              \ Volt=Amplitude*[Exp(B(time))]+Constant
Real Scalar Constant
```

```

Real Dim[ 3 ] Array Fit.coeffs
15 Max.#.Iterations :=
.0005 Fit.tolerance :=
\
\ *****DECLARATION OF TEMPLATES*****
\
3 3 A/d.template V1a 106 Template.repeat V1aoutput Template.buffer
3 3 A/d.template V1b 151 Template.repeat V1boutput Template.buffer
\
0 0 D/a.template Vapp          \ Applied Voltage Template
\
1 1 D/a.template Vheat         \ Heater Voltage Template
\
\ *****DECLARATION OF STRINGS*****
\
20 String File.name           \ File name to save data
\
\ *****PROGRAM WORDS*****
: Apply.bridge.voltage
  Normal.display
  ." Input bridge voltage (0-2 V) "
  #Input
  Vapplied :=
  Vapp D/a.init Vapplied -5.0 5.0 D/a.scale D/a.out
;
\ Set default bridge voltage
  0.65 Vapplied :=
\
: Apply.heater.voltage
  Normal.display
  ." Input switch voltage to turn on (1.25) off (0) heater"
  #Input
  Vheater :=
;
\ Set Default heater voltage
  1.25 Vheater :=
\
: Read.Therm.Volt
  16.667 Conversion.delay
  Vheat D/a.init Vheater -5.0 5.0 D/a.scale D/a.out \ Heat on
  V1a 2.0 a/d.gain A/d.init A/d.in>array
  Vheat D/a.init 0 -5.0 5.0 D/a.scale D/a.out      \ Heat off
  16.667 Conversion.delay
  V1b 2.0 a/d.gain A/d.init A/d.in>array          \ Take Data
;
\
: Get.Therm.Volt
  Read.Therm.Volt
  V1boutput Sub[ 2 , 150 , ! ]
  -2.5 2.5 A/d.scale 5.0 / Temp1.curve :=
;
Time1 []Ramp Time1 16.667 * 1000. / Time :=

```

```

\
: Plot.Data
  CR CR
  ." Plot Thermistor Data (1) or Return to Menu (2) ? "
  #Input
\
  Case
    1 of  Time1 Temp1.curve xy.auto.plot Endof
    2 of  Nop                               Endof
  Endcase
;
\
: Save.data
  File.template
  2 Comments
  Real Dim[ 150 ] Subfile
  Real Dim[ 3 ] Subfile
  End
\
  Cr ." Enter Filename to be Saved : "
  "Input File.name " :=
  File.name Defer> File.create File.name File.open
  " Filename: " File.name "Cat "   Date:" "Date "Cat "Cat
  1 >Comment
  " Not fitted " 2 >Comment
  1 Subfile Temp1.curve Array>file
  File.close
;
\
: Print.data
  Console.off
  Out>printer
  Cr ." File.name: " File.name "type
  Cr ." Date      : " "date "type
  Cr ." Voltages" Cr
  Temp1.curve .
  Time1 Temp1.curve Xy.auto.plot
  Outline
  Screen.Print
  Console
;
\
  Initial Guesses for fit routine
.15 Amplitude :=
-1.0 Bexp :=
.28 Constant :=
\
  Fitting Function
: Func
  Bexp * exp Amplitude * Constant +
;
\

```

```

: Find.Fit.Parameters
  Curve.Fit[ time1 , temp1.curve ; Func ; Amplitude , Bexp , Constant ]
  Drop
;
\
\   Fit current data
: Fit.same.file
  Solid   Time1 Temp1.curve Xy.auto.plot
          Find.fit.parameters
          Cr Amplitude .
          Cr Bexp .
          Cr Constant .
          cr
          .01 .01 .01 .01 Dashed Time1 Time1 Func Xy.data.plot Solid
;
\
\   Fit data from another file
: Fit.other.file
  Cr ." Enter Filename "
  "Input File.name " :=
  File.name Defer> File.open
  1 subfile Temp1.curve File>array
  File.close
  Fit.same.file
;
\
: Save.fit.parameters
  Amplitude Fit.coeffs [ 1 ] :=
  Bexp      Fit.coeffs [ 2 ] :=
  Constant  Fit.coeffs [ 3 ] :=
  File.name Defer> File.open
  " Fitted " 2 >Comment
  2 Subfile Fit.coeffs Array>file
  File.close
;
\
: Parameter.Routine
  Cr Cr
  ." 1           Fit current data "           Cr
  ." 2           Fit data on file "           Cr
  ." 3           Save fit parameters in file " Cr
  ." 4           Return to Main Menu " Cr
  Cr
  ."           Enter Number "
  #Input

  Case 1 of Fit.same.file Myself      Endof
    2 of Fit.other.file Myself        Endof
    3 of Save.fit.parameters Myself   Endof
    4 of Nop                          Endof
  Endcase
;

```



```

\
: Start
  Normal.display
  Cr Cr ."          Thermal Probe Program " Cr CR
  Cr Cr
  ." 1.      Turn off/on heater (default on)"  Cr
  ." 2.      Take.data"  Cr
  ." 3.      Plot Data "      Cr
  ." 4.      Save Data "      Cr
  ." 5.      Take and Save Data "  Cr
  ." 6.      Print Data & Curve " Cr
  ." 7.      Fit Data
  ." 9.      Quit "      Cr
  Cr
  ."          Enter Number "
  #Input
\
  Case
    1 of  Apply.heater.voltage      Endof
    2 of  Get.Therm.Volt            Endof
    3 of  Plot.Data                  Endof
    4 of  Save.data                  Endof
    5 of  Get.Therm.Volt Save.data    Endof
    6 of  Print.data                 Endof
    7 of  Parameter.Routine          Endof
    9 of  Abort                      Endof

  Endcase
  Myself
;
  Vapp D/a.init Vapplied -5.0 5.0 D/a.scale D/a.out
  Vheat D/a.init 0 -5.0 5.0 D/a.scale D/a.out
\
Start

```

Appendix D

Standard Surgical Protocol

Female Yorkshire pigs weighing between 30–40 kg, were immobilised with intramuscular injections of valium (1 cc) and ketamine (20 mg/kg) with (1 cc) atropine. To minimize stress and handling of the animal, a long lead with the needle at one end and the syringe at the other was used to deliver this premedication. With the animal in dorsal recumbency, a 50/50 oxygen/nitrogen mixture with 3% halothane was used to induce anaesthesia. After intubation and mechanical ventilation, the anaesthesia was reduced to 1%. Fluid balance was maintained with 5000 UPS/L heparinized saline administered intravenously at a rate of 2–4 ml/hr/kg. Three ECG leads were attached and an oesophageal temperature probe was inserted to monitor core body temperature. The left ventricle was cannulated with a French #7 pig tail catheter to monitor ventricular pressure and to inject microspheres if required. Central venous and left femoral pressure were also monitored as required. The bladder was cannulated to measure total urine output. A long ventral midline incision from the xiphoid to approximately 10 cm below the umbilicus was made and the left kidney was exposed by displacing the bowels to the right side. The viscera were covered with moist surgical towels. The kidney was deflected laterally and the renal capsule was dissected away from the ventral surface. The renal capsule was clamped to the body wall or sutured to long weighted clamps to help stabilize the kidney and sustain the bowels in their retracted positions. Note that the left kidney is more distal than the right and that the right is cranial to the left.

END

24/1 2/92

FIN